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# CONTENTS OF VOLUME 30

## FEBRUARY, 1944, NUMBER 1.

Cort, W. W., D. J. Ameal and Louis Olivier. AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF <i>Schistosomatium douthitti</i> (CORT, 1914) IN ITS INTERMEDIATE HOST .....	1
Larsh, John E. Jr. and Alan W. Donaldson. THE EFFECT OF CONCURRENT INFECTION WITH <i>Nippostrongylus</i> ON THE DEVELOPMENT OF <i>Hymenolepis</i> IN MICE .....	18
Larsh, John E. Jr. COMPARATIVE STUDIES ON A MOUSE STRAIN OF <i>Hymenolepis nana</i> VAR. <i>fraterna</i> , IN DIFFERENT SPECIES AND VARIETIES OF MICE .....	21
Jones, Myrna F. and Alexander Hollaender. EFFECT OF LONG ULTRAVIOLET AND NEAR VISIBLE RADIATION ON THE EGGS OF THE NEMATODES <i>Enterobius vermicularis</i> AND <i>Ascaris lumbricoides</i> .....	26
RESEARCH NOTES.	
Hogue, M. J. <i>Trichomonas vaginalis</i> IN THE FLUID OF THE VAGINA .....	34
Brady, Frederick J. and Alfred H. Lawton. A NEW METHOD FOR QUANTITATIVE ESTIMATION OF MICROFILARIAE IN BLOOD SAMPLES .....	34
Young, Viola Mae and Oscar Felsenfeld. THE INCIDENCE OF <i>Embodomonas intestinalis</i> WENYON AND O'CONNOR IN FOOD HANDLERS AND DIARRHEIC PATIENTS OF MENTAL HOSPITALS .....	34
Fischthal, Jacob H. OBSERVATIONS ON A SPOROZOAN PARASITE OF THE EELPOUT, <i>Zoarces anguillar</i> , WITH AN EVALUATION OF CANDLING METHODS FOR ITS DETECTION .....	35

## APRIL, 1944, NUMBER 2.

Cort, W. W. and D. J. Ameal. FURTHER STUDIES ON THE DEVELOPMENT OF THE SPORO-CYST STAGES OF PLAGIORCHID TREMATODES .....	37
Boardman, Edward T. METHOD FOR COLLECTING TICKS FOR STUDY AND DELINEATION ..	57
Hardcastle, A. B. <i>Eimeria brevoortiana</i> , A NEW SPOROZOAN PARASITE FROM MENHADEN ( <i>Brevoortia tyrannus</i> ), WITH OBSERVATIONS ON ITS LIFE HISTORY .....	60
Olivier, Louis. ACQUIRED RESISTANCE IN CHICKENS, TURKEYS, AND RING-NECKED PHEASANTS TO THE GAPEWORM, <i>Syngamus trachea</i> .....	69
Cooley, R. A. and Glen M. Kohls. THE GENUS <i>Amblyomma</i> (IXODIDAE) IN THE UNITED STATES .....	77
Herman, Carlton M. NOTES ON THE PUPAL DEVELOPMENT OF <i>Stilbometopa impressa</i> (DIPTERA: HIPPOBOSCIDAE) .....	112
Goble, Frans C. and E. L. Cheatum. NOTES ON THE LUNGWORMS OF NORTH AMERICAN LEPORIDAE .....	119
Brand, Theodor von and W. F. Simpson. PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL <i>Eustrongylides</i> . VII. STUDIES UPON SURVIVAL AND METABOLISM IN STERILE SURROUNDINGS .....	121
AMERICAN SOCIETY OF PARASITOLOGISTS. PRELIMINARY ANNOUNCEMENT OF THE NINETEENTH ANNUAL MEETING .....	130

## JUNE, 1944, NUMBER 3.

Armer, Sister Joseph Marie. INFLUENCE OF THE DIET OF BLATTIDAE ON SOME OF THEIR INTESTINAL PROTOZOA .....	131
Harwood, Paul D. and James E. Guthrie. THE EFFECT OF NICOTINE-BENTONITE AND OF CERTAIN PHYSICAL STATES UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS .....	143
Fujii, Harold. THREE MONOGENETIC TREMATODES FROM MARINE FISHES .....	153
Chen, H. T. <i>Spelotrema pseudogonotyla</i> n. sp. (TREMATODA: MICROPHALLIDAE) FROM HONGKONG .....	159
Hauschka, Theodore. SEASON, NUTRITION, IMMUNITY, DRUGS AND X-RAY AS FACTORS INFLUENCING THE COURSE OF A COCCIDIAN INFECTION .....	162
Stabler, Robert M. A NEW SPECIES OF <i>Retortamonas</i> (PROTOZOA) FROM THE COMMON MOLE CRICKET, <i>Gryllotalpa hexadactyla</i> .....	173
Simpson, Myron L. EXOERYTHROCYTIC STAGES OF <i>Plasmodium durae</i> .....	177
Witenberg, G. <i>Transversotrema haasi</i> , A NEW FISH TREMATODE .....	179
Keegan, Hugh L. ON A NEW GENUS AND SPECIES OF PARASITID MITE .....	181

Nigrelli, Ross F. and L. W. Maraventano. PERICARDITIS IN <i>Xenopus laevis</i> CAUSED BY <i>Diplostomulum xenopi</i> sp. nov., A LARVAL STRIGEID .....	184
Martin, W. E. <i>Cercaria solemyae</i> n. sp., PROBABLY A BLOOD FLUKE, FROM THE MARINE PELECYPOD, <i>Solemya velum</i> .....	191
Meleney, Henry E. WAR-TIME ACTIVITIES OF MEMBERS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS .....	195
RESEARCH NOTES.	
Harwood, Paul D. and James E. Guthrie. THE EFFECT OF PRICKLY-ASH BARK UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS' .....	197
Wood, Sherwin F. NOTES ON THE FEEDING OF CONE-NOSED BUGS (HEMIPTERA: REDUVIIDAE) .....	197
Hauschka, T. S. and R. B. Doll. <i>Paraglaucoma</i> sp., A FACULTATIVE PARASITE OF COELENTERATES .....	198
Wood, Sherwin F. AN ADDITIONAL CALIFORNIA LOCALITY FOR <i>Trypanosoma cruzi</i> CHAGAS IN THE WESTERN CONE-NOSED BUG, <i>Triatoma protracta</i> (UHLER) .....	199
Sullivan, Thelma D. VIABILITY OF <i>Trypanosoma cruzi</i> IN CITRATED BLOOD STORED AT ROOM TEMPERATURE .....	200
Doetschman, Willis H. NOTES ON ANOPLURA INFESTING MARINE CARNIVORES .....	200
Riley, William A. THE OCCURRENCE OF <i>Amblyonema americanum</i> IN MINNESOTA AND IN OHIO .....	200
Yetwin, I. Jacques. A SIMPLE PERMANENT MOUNTING METHOD FOR <i>Necator americanus</i> .....	201
Reinhard, Edward G. A HERMIT CRAB AS INTERMEDIATE HOST OF <i>Polymorphus</i> (ACANTH.) .....	201
Stabler, Robert M. <i>Giardia sanguinis</i> (GONDER, 1911) NOT FROM A "FALCON" .....	202
Gates, Doris B. <i>Xenopsylla cheopis</i> IN LINCOLN, NEBRASKA .....	202
AMERICAN SOCIETY OF PARASITOLOGISTS. MINUTES OF 32ND COUNCIL MEETING, BALTIMORE, MD., JANUARY 29, 1944 .....	203
SECOND REPORT OF THE COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA .....	206

## AUGUST, 1944, NUMBER 4.

Naiman, Dorothy Neuhof. EFFECT OF X-IRRADIATION OF RATS UPON THEIR RESISTANCE TO <i>Trypanosoma lewisi</i> .....	209
Addis, C. J. and Asa C. Chandler. STUDIES ON THE VITAMIN REQUIREMENT OF TAPE-WORMS .....	229
Augustson, Gustaf F. THE FLEA GENUS <i>Thrassis</i> AND SYLVATIC PLAGUE, WITH THE DESCRIPTION OF <i>T. brennani</i> n. sp. ....	237
Davis, Helen Edith. <i>Cittotacnia sandgroundi</i> , A NEW ANOPLCEPHALID CESTODE FROM A JAVANESE TREE DUCK .....	241
Witenberg, G. and Ch. Gerichter. THE MORPHOLOGY AND LIFE HISTORY OF <i>Foleyella duboisi</i> WITH REMARKS ON ALLIED FILARIDS OF AMPHIBIA .....	245
Ameel, Donald J. THE LIFE HISTORY OF <i>Nudacotyle novicia</i> BARKER, 1916 (TREMATODA: NOTOCOTYLIDAE) .....	257
Anderson, Dorcas J. STUDIES ON <i>Cercaria szidati</i> sp. nov., A NEW FURCOCEROUS CERCARIA OF THE VIVAX TYPE .....	264
Todd, A. C. TWO NEW NEMATODES FROM THE AQUATIC BEETLE <i>Hydrous triangularis</i> (SAY) .....	269
RESEARCH NOTES	
Chandler, Asa C. A NEW SPECIES OF <i>Mesocestoides</i> , <i>M. kirbyi</i> , FROM <i>Canis latrans</i> .....	273
James, Maurice T. TWO ERRONEOUS RECORDS IN AMERICAN LITERATURE OF THE CAUSATIVE AGENTS OF MYIASIS .....	273
Rozeboom, L. E. <i>Phlebotomus limai</i> FONSECA IN THE UNITED STATES (DIPTERA: PSYCHODIDAE) .....	274
Zeliff, C. Courson. <i>Barroella</i> n. nom. FOR <i>Kirbyella</i> ZELIFF, 1930, HOMONYM .....	275

## AUGUST SUPPLEMENT, 1944.

AMERICAN SOCIETY OF PARASITOLOGISTS.	
PROGRAM, 19TH ANNUAL MEETING, CLEVELAND, OHIO, SEPT. 11-12, 1944. ....	1
AUTHOR INDEX .....	4
PROGRAM, SYMPOSIUM ON PARASITOLOGY IN RELATION TO THE WAR .....	5
ABSTRACTS .....	6
OFFICERS .....	19
IN MEMORIAM .....	22
LIST OF NEW MEMBERS .....	23

## OCTOBER, 1944, NUMBER 5.

Denton, J. Fred. STUDIES ON THE LIFE HISTORY OF <i>Eurytrema procyonis</i> DENTON, 1942 ..	277
Cooley, R. A. <i>Ixodes ozarkus</i> n. sp. AND <i>Ornithodoros aquilae</i> n. sp., WITH NOTES ON <i>O. talaje</i> AND <i>O. kelleyi</i> (IXODOIDEA) ..	287
Smith, B. F. and C. A. Herrick. THE RESPIRATION OF THE PROTOZOAN PARASITE, <i>Eimeria tenella</i> ..	295
Senekjic, Harry A. AMERICAN VISCERAL LEISHMANIASIS—THE ETIOLOGICAL AGENT ....	303
Cort, W. A., Sterling Brackett and Louis Olivier. LYMNAEID SNAILS AS SECOND INTERMEDIATE HOSTS OF THE STRIGEID TREMATODE, <i>Cotylurus flabelliformis</i> (FAUST, 1917) ..	309
Wenrich, D. H. STUDIES ON <i>Dientamoeba fragilis</i> (PROTOZOA). IV. FURTHER OBSERVATIONS, WITH AN OUTLINE OF PRESENT-DAY KNOWLEDGE OF THIS SPECIES ..	322

## DECEMBER, 1944, NUMBER 6.

Ewing, H. E. THE TROMBICULID MITES (CHIGGER MITES) AND THEIR RELATION TO DISEASE ..	339
Augustson, Gustaf F. A NEW MOUSE FLEA, <i>Pleochaetoides bullisi</i> , n. gen., n. sp., FROM TEXAS ..	366
Van Cleave, Harley J. and Elizabeth L. Ross. PHYSIOLOGICAL RESPONSES OF <i>Neoechinorhynchus emydis</i> (ACANTHOCEPHALA) TO VARIOUS SOLUTIONS ..	369
Hawkins, Philip A., C. L. Cole and E. E. Kline. STUDIES OF SHEEP PARASITES. IV. SURVIVAL OF SHEEP NEMATODES ON PASTURE DURING THE FALL MONTHS ..	373
Bushland, R. C., L. C. McAlister, Jr., G. W. Eddy, Howard A. Jones and E. F. Knippling. DEVELOPMENT OF A POWDER TREATMENT FOR THE CONTROL OF LICE ATTACKING MAN ..	377
BOOKS AND MONOGRAPHS RECEIVED ..	388
AMERICAN SOCIETY OF PARASITOLOGISTS.	
MINUTES OF THE ANNUAL AND COUNCIL MEETINGS ..	390
INDEX FOR VOLUME 30, NUMBERS 1-6 ..	393
INDEX FOR AUGUST SUPPLEMENT ..	398





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Number 1

## AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF *SCHISTOSOMATIUM DOUTHITTI* (CORT, 1914) IN ITS INTERMEDIATE HOST<sup>1</sup>

W. W. CORT, D. J. AMEEL,<sup>2</sup> AND LOUIS OLIVIER<sup>3</sup>

In spite of all the investigations that have been made on schistosome life cycles, surprisingly little is known of the development of the sporocyst stages and the germ cell cycle in this group. In a recent paper (Cort and Olivier, 1943a) results were reported of studies on natural infections of a member of this group, *Cercaria stagnicola* Talbot, 1936, which have given some new information on these phases of development.

The present paper gives the results of studies on the sporocyst stages of *Schistosomatum douthitti* from experimental infections of snail intermediate hosts. All the observations were made on living material of mother and daughter sporocysts in infections of different ages (for methods, see Cort and Olivier, 1941).

The miracidia used in the experimental infections were obtained from eggs passed by laboratory mice which had been infected with cercariae of *S. douthitti* from natural infections in *Lymnaea stagnalis appressa* Say and *Lymnaea stagnalis perampla* Walker. In the summer of 1941 small juveniles of *Stagnicola emarginata angulata* (Sowerby) collected from a lake near the Biological Station were used for the experimental infections. This snail is obviously not a natural intermediate host for *S. douthitti*, since in the thousands that have been examined from the region only one infection has been found (Cort et al., 1937). During the summer of 1941 only mother sporocysts, not including the youngest stages or those that were fully developed, were obtained. We, therefore, returned to the study in the summer of 1942, using laboratory raised juveniles of a natural intermediate host, *Stagnicola palustris elodes* (Say). With an abundant supply both of the snails and infected mice, we were able to study the stages in the development of *S. douthitti* from very young mother sporocysts to mature daughters.

Time was not available for attempting single miracidium infections in the experimental snails, so that groups of them were infected in aquaria of various sizes simply by putting in fecal material containing the eggs or by pouring in miracidia that had hatched. In the summer of 1941 large numbers of eggs in fecal pellets were intro-

<sup>1</sup> A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is one of a series on the embryology of digenetic trematodes in the intermediate hosts. The others have dealt with another schistosome (Cort and Olivier, 1943a), the strigeids (Cort and Olivier, 1941), and a plagiurchiid (Cort and Olivier, 1943b).

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duced over a period of about a week into an aquarium containing about 100 juveniles of *S. c. angulata* resulting in the infection of almost all the snails; in fact, multiple infections were the rule with some snails having as many as 5 or 6 mother sporocysts. In the summer of 1942 all the miracidia used in infecting the juveniles of *S. p. elodes* in a given aquarium were allowed to hatch and were introduced at one time, so that it was possible to know the age of the infections produced. In the experimental infections of this summer the majority of the snails were infected, but multiple infections appeared to be rare. The mother sporocysts were not difficult to find except in the very youngest stages, since as reported by Price (1931) they are localized in the snails, being found in the tissues close to the beginning of the esophagus near the cerebral ganglia.

DEVELOPMENT OF THE SPOROCYSTS OF *S. douthitti* IN EXPERIMENTALLY  
INFECTED SNAILS

For convenience in description the development of *S. douthitti* in the intermediate host can be divided into three phases, viz., (1) the germ cell multiplication in the young mother sporocyst, (2) the embryonic development of the daughter sporocysts within the mother, and (3) the development of the daughter sporocysts in the tissues of the snail intermediate host.

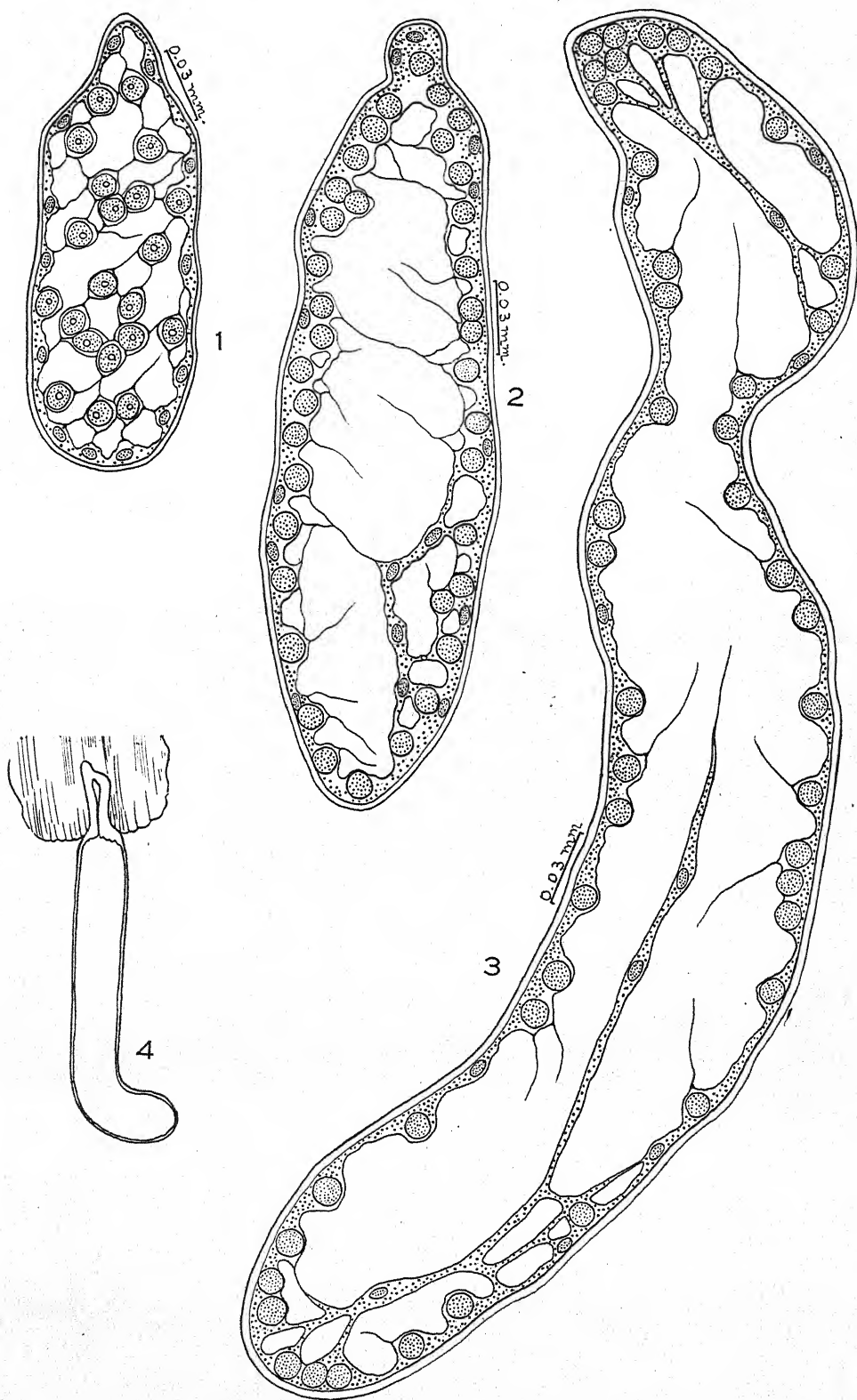
*Germ Cell Multiplication in the Young Mother Sporocysts*

Five very young mother sporocysts were found in one group of *S. p. elodes* about 65 to 69 hours after exposure to the miracidia. They varied in length from about 0.12 to 0.20 mm and in width from about 0.05 to 0.08 mm. They had, therefore, grown only slightly since Price (l. c. p. 700) gave the average size of heat killed miracidia of *S. douthitti* as 0.131 by 0.060 mm. When these young mother sporocysts were freed from the snail's tissues, they were able to extend and contract the body with a slight wriggling movement, brief periods of rest being followed by periods of activity, which, however, did not result in any locomotion. The ciliated epithelium of the miracidium had been lost, and in two cases masses of material next to the sporocysts appeared to be remnants of this layer. Fig. 1 shows a drawing in optical section (camera lucida outline) of one of these very young mother sporocysts. All the structures that fill the anterior half of the miracidium (rudimentary digestive tract, penetration glands and nerve mass) have completely disappeared. The body wall is of uniform thickness and structure and the anterior end can be distinguished only by its greater mobility and attenuation. The inner layer of the body wall has a greyish granular appearance from numerous small globules in its cytoplasm and at intervals shows slight projections with the small oval nuclei. The germ cells are scattered throughout the body cavity either singly or in groups and are attached by cytoplasmic strands to the inside of the wall and to each other. Price (l. c. fig. 13) in an optical section of a miracidium of *S. douthitti* showed numerous germ cells (26 in all) with large nuclei, prominent nucleoli and a thin layer of cytoplasm with fiber-

FIGS. 1, 2, and 3. Very young mother sporocysts, in optical section, from experimental infections in laboratory raised juveniles of *S. p. elodes*. Fig. 1 about 65 hours and figs. 2 and 3 about 110 to 116 hours after infection. The constricted region in figure 3 near the anterior end was due to handling.

FIG. 4. Outline drawing of young mother sporocyst showing attachment to the snail's tissue by the attenuated anterior end. Size, 0.7 mm by 0.07 mm.





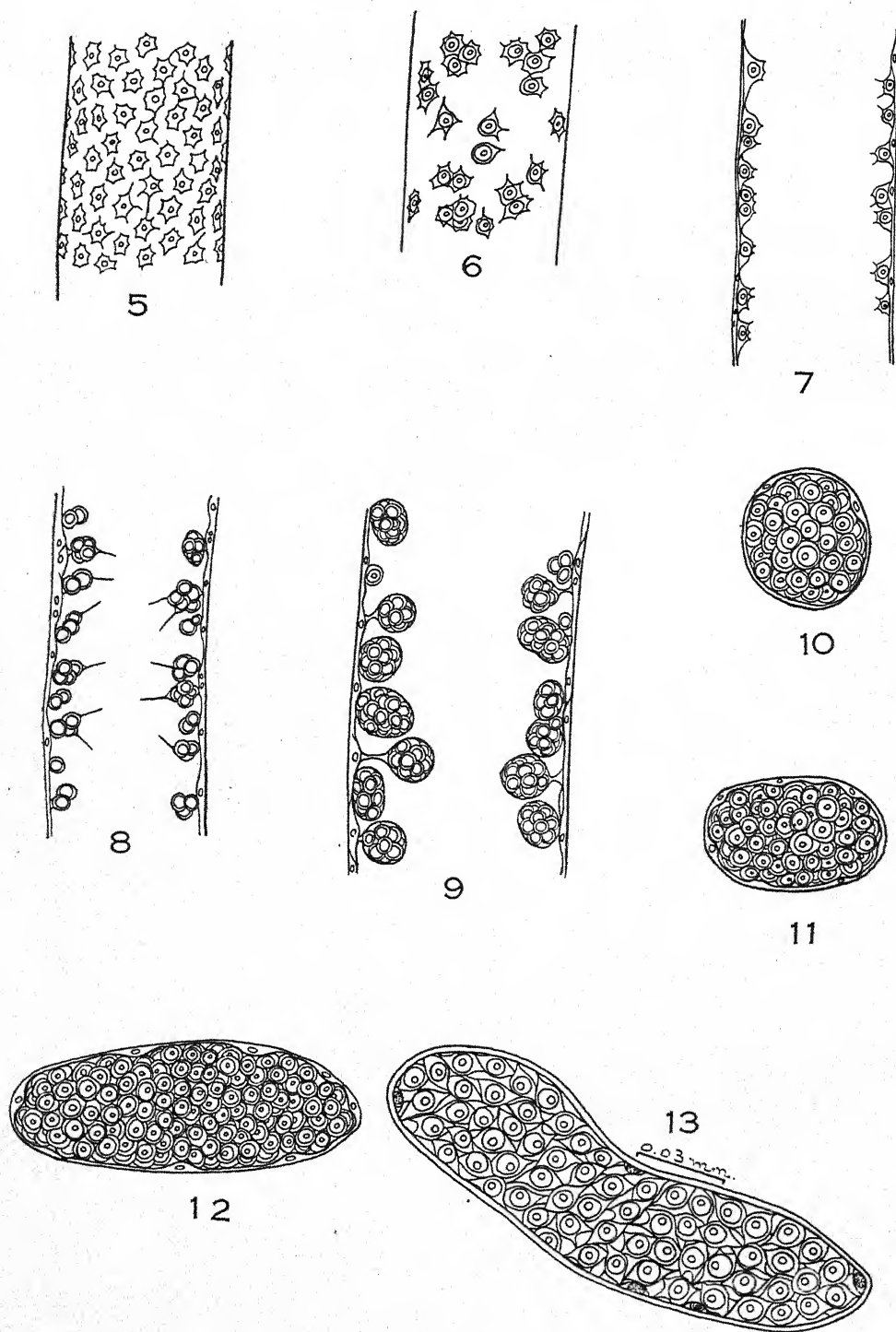


FIG. 5. Surface view of somewhat contracted young mother sporocyst showing the structure of the cells of the body wall. About 0.10 mm in width.

FIG. 6. View with focus just below wall of young mother sporocyst showing arrangement of undivided germ cells beneath the cellular layer of the wall. Scale about that of figure 5.

like processes connecting with the body wall. The germ cells of our youngest mother sporocysts had the same structure as those of the miracidium. Counts of these germ cells, which were probably under the actual number, ranged from about 30 to 40, suggesting that as yet there had been little, if any, increase in number.

Young mother sporocysts obtained from snails (*S. p. elodes*) 110 to 116 hours after exposure showed considerable further growth and development and a much greater variation in size. The germ cells had increased in number and were distributed along the inside of the body wall. This left a large central body cavity crossed by a few strands some of which contained nuclei. The nuclei of the germ cells showed no nucleoli as in the earlier stage, which suggested that they were in a phase of multiplication. Fig. 2 shows an optical section of one of the smallest of the mother sporocysts of this age, which has a size of about 0.30 mm by 0.09 mm. There is a distinct anterior tip enclosed in the host's tissue. The body wall appears thicker and somewhat more granular than in the earlier stage and the nuclei somewhat more scattered. The number of germ cells was estimated after repeated attempts to count them at somewhat less than 100. Fig. 3 shows the largest mother sporocyst found in the group of snails examined at 110 to 116 hours. The width is about the same as that of the specimen shown in Fig. 2 but the length is much greater (almost 0.6 mm). The inner layer of the body wall appears somewhat thinner and its nuclei are more scattered; the germ cells are more scattered even though they have increased in number; more than 150 were counted in this specimen. A few strands from the body wall, some containing nuclei, traverse the lumen of the enlarged body cavity.

After the stage shown in Fig. 3 there appears to be some further increase in the number of the germ cells of the young mother sporocysts. In most cases the germ cells begin to develop into embryos in mother sporocysts which are about a week old, although in the 1941 infections some at least two weeks old and 1.2 mm in length were found with no developing embryos. Fig. 4 shows the shape and method of attachment to the snail's tissue of a young mother sporocyst at this stage. In the largest mother sporocysts containing only germ cells the nucleoli of these cells can be clearly seen (Fig. 7) suggesting that multiplication had been completed. The body wall appears to be slightly thinner and more opaque than in the earlier stages; the nuclei of its inner layer are still easily seen in slight projections into the body cavity. They belong to cells of the body wall which appear in surface view as scattered stellate cells with numerous cytoplasmic processes as shown in Fig. 5 in a somewhat contracted specimen. Fig. 6, a view with the focus just below the body wall, shows the appearance of the germ cells from surface view. If the relation of

FIG. 7. Optical section of a small part of a young mother sporocyst one to two weeks after infection, showing the relation of the germ cells to the body wall. Scale about that of figure 5.

FIG. 8. Optical section of somewhat older stage than figure 7, showing the relation of the early cleavage stages of the daughter sporocyst embryos to the body wall. About same scale as figure 5.

FIG. 9. Optical section showing the relation of the developing daughter sporocyst embryos (round "germ ball" stage) to the body wall. Width about 0.15 mm. Figures 5-9 were drawn from young daughter sporocysts from experimental infections in juveniles of *S. e. angulata*.

FIG. 10. Daughter sporocyst embryo in slightly later stage than those shown in figure 9. Diameter 0.050 mm.

FIGS. 11 and 12. Daughter sporocyst embryos showing early stages of elongation. Fig. 11, size 0.064 by 0.040 mm; Fig. 12, size 0.136 by 0.057 mm.

FIG. 13. Daughter sporocyst embryo slightly more advanced than in fig. 12, showing cytoplasmic projections from germ cells.

the germ cells in the miracidium and youngest mother sporocysts (Fig. 1) had not been known, it might easily have been thought that they developed in the inner layer of the sporocyst wall; for especially in contracted specimens they appear to form an inner layer of the wall (Fig. 7).

*Development of Daughter Sporocyst Embryos Inside the Mother*

Fig. 8 shows the early cleavage stages of the development of the embryos inside somewhat older mother sporocysts. At this stage, the cell groups are still attached closely to the inside of the body wall by protoplasmic strands, but they are more definitely separated from the wall than the germ cells of the earlier stages (cf. Figs. 7 and 8). In some sporocysts that were found with 2 to 8 cell cleavage stages, only a few single germ cells would be left; in others about half the germ cells would not have started development. Mother sporocysts with the germinal material at this stage varied in length from less than 1 mm to about 2.5 mm.

As development continued more and more of the mother sporocysts were found with embryos in the "germ ball" stage (Fig. 9). Such embryos are still attached to the inside of the wall, but stand out further into the body cavity. Along with the round "germ balls" a few germ cells and early cleavage stages are still present although most of the embryos are usually in about the same stage of development. During this stage of early development of the daughter sporocyst embryos, the young mother sporocysts continue to grow rapidly; also, their variation in size at any given age becomes greater. In one mother sporocyst of the 1941 series, 3.5 mm in length the embryos were almost all in this early round "germ ball" stage varying in diameter from 0.02 to 0.05 mm. In a 13-day old mother sporocyst of the 1942 series, which was 4.7 mm in length by 0.26 mm in width, the embryos were also in the round "germ ball" stage, the largest measuring 0.07 mm.

The embryo in the round "germ ball" stage (about 0.03 to 0.07 mm in diameter) consists of a mass of tightly packed cells surrounded by a thin membrane in which a few flattened nuclei can be seen (Fig. 10). From this outer membrane develops the body wall of the daughter sporocyst. The enclosed cells are typical germ cells like those of the mother sporocyst, with large nuclei, prominent nucleoli and a small amount of cytoplasm. They are so closely packed at this stage that it was impossible to determine whether they had cytoplasmic processes connecting them with the body wall or with each other.

After the embryos in the "germ ball" stage reach a diameter of about 0.05 to 0.07 mm they begin to elongate (Figs. 11 and 12). They are still attached to the inside of the body wall of the mother sporocyst and a few earlier stages and even single germ cells can still be found. In these elongate daughter sporocyst embryos, the wall appears thicker and the germ cells more numerous. Occasionally at this stage it was possible by careful observation to see cytoplasmic processes extending from the germ cells. Mother sporocysts containing embryos that had started to elongate had increased considerably in size. One, 13 days old and 8.4 mm in length, had most of its embryos showing elongation, the largest having a size of 0.11 by 0.09 mm. Another still larger mother (11.4 by 0.29 mm) found 15 days after infection contained still larger elongate embryos, the largest have a size of 0.12 by 0.09 mm. In these specimens the embryos were still attached to the wall; in fact, in one mother sporocyst, embryos that had begun to take the form of daughter sporocysts, which



were about the stage of Fig. 13 and almost 0.15 mm in length, were still attached to the wall.

At the stages of development just described it was possible to make some fairly accurate counts of the numbers of embryos present in individual mother sporocysts. One, 11.4 mm long, contained 172 embryos; in another 4.7 in length there were 206, and for a third 4.7 mm long the total count reached 420.

After the daughter sporocyst embryos reached the stage shown in Fig. 13 the germ cells usually appear slightly less crowded and cytoplasmic processes connecting them with the wall and with each other can usually be seen on careful examination. The nuclei of the body wall can be made out as projections into the body cavity. After this stage the daughter sporocyst embryos increase rapidly in length and begin to show activity. About this time, also, the connections of the embryos to the body wall of the mother sporocysts are broken down and they are found floating free in the body cavity. From this stage until the first daughters are ready to escape from the mother the growth in length of the daughter sporocysts proceeds rapidly. In an extraordinarily large mother sporocyst (26.6 mm in length) found 17 days after infection, almost all the daughter sporocyst embryos were distinctly elongate, varying from 0.11 mm by 0.08 mm to 0.45 by 0.08 mm. The largest showed considerable activity but had not yet reached the migrating stage.

A daughter sporocyst embryo which had almost completed its development and is approaching the migrating stage is shown in Fig. 14. Such daughter sporocysts are beginning to show considerable activity, indicating that the muscle layers of the wall are well developed. Some nuclei of the inner layer of the body wall can still be seen. The germ cells are usually not nearly so crowded as in earlier stages and their cytoplasmic projections can almost always be easily seen. There is, however, some variation in this respect, since in some mother sporocysts, daughters that appear almost ready to migrate have the germ cells still crowded, and their cytoplasmic processes can be seen only with difficulty.

By 20 days after infection most of the mothers contained some daughter sporocysts that appeared fully developed and ready to migrate. In one mother 20½ days old large numbers of active daughters were present that varied in length from 0.38 to 0.83 mm; in another of the same age there were large numbers of daughters that appeared ready to escape and only eight smaller embryos. In neither of the snails in which these two mothers were found were there any daughters outside the mothers.

The structure of a daughter sporocyst that appeared ready to migrate from the mother is shown in Fig. 15. Such daughters, the largest of which are slightly under 1 mm in length, are very active and can extend and contract the body greatly. The anterior end is distinctly pointed and covered with small backward-pointing spines. No attempt was made to work out the details of structure of the body wall. Evidently muscular layers are well developed. Occasional nuclei can still be made out in the inner layer of the wall which has become quite opaque from the numerous globules which it contains. In the specimen shown in Fig. 15 the germ cells are already loosely scattered in the body cavity and are held in place by their cytoplasmic processes. A few of them have already developed into embryos which are held in position by strands connecting with the wall. This represents a precocious development of the germ cells, since in the majority of fully developed daughters observed

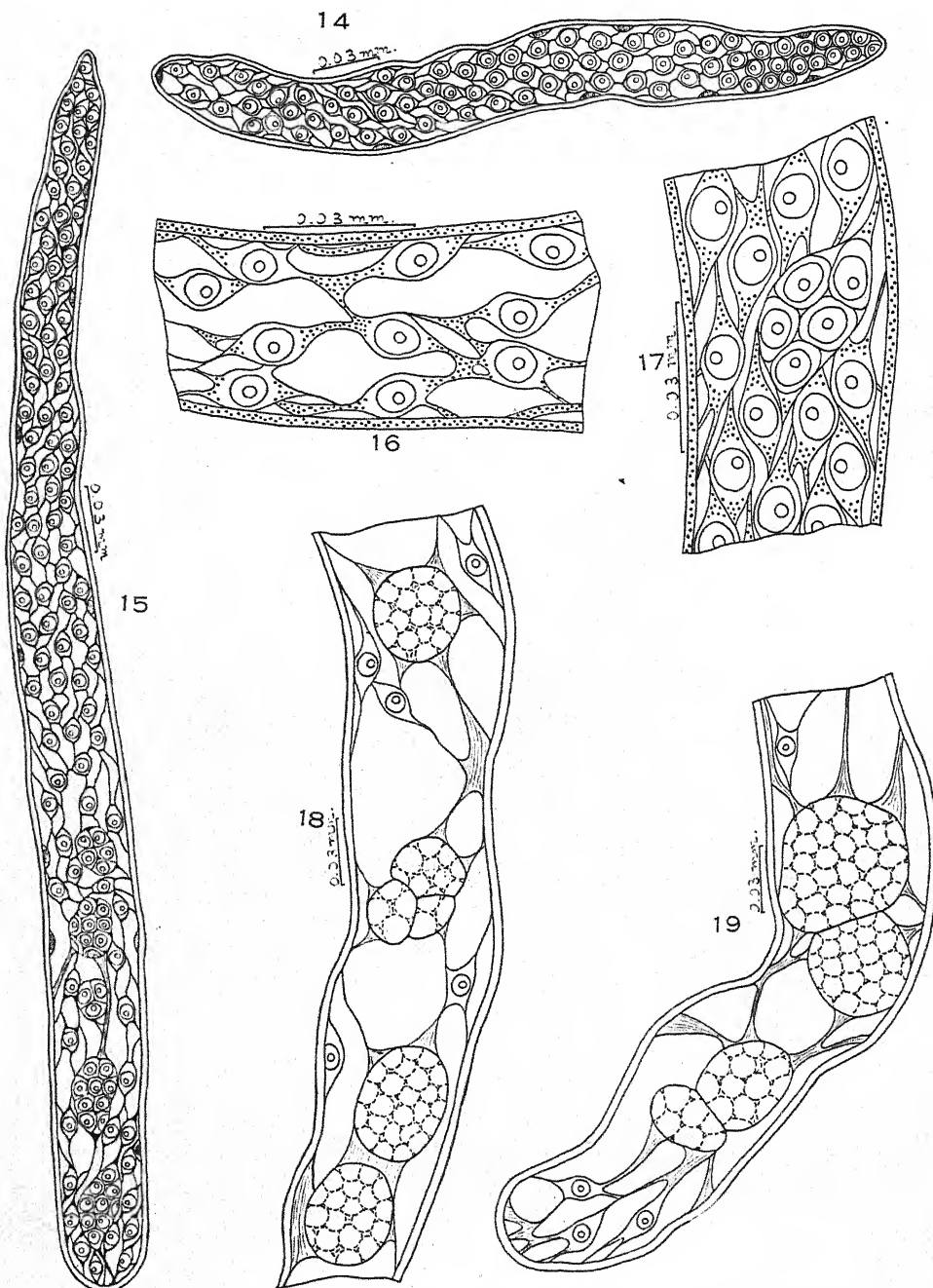


FIG. 14. Daughter sporocyst embryo, just before migrating stage, from a 17-day-old mother sporocyst.

FIG. 15. Daughter sporocyst which has completed its development and appears ready to migrate from a 20½-day-old mother.

FIG. 16. Optical section of a small part of a migrating daughter sporocyst 1.7 mm long from the digestive gland of a snail 21½ days after infection. In this case none of the germ cells had started to develop although they were separated by spaces on account of the rapid growth of the sporocyst.

inside of the mothers only germ cells were present, which were usually closer together than in the sporocyst shown in Fig. 15. In fact, in a few cases daughters outside the mothers, which were considerably longer than any found still inside the mother sporocysts, contained no developing embryos. One such daughter, which was 2.06 mm long, contained about 238 germ cells and no developing embryos. On the other hand, precocious development of the embryos may be even greater than shown in Fig. 15. In a 17-day old mother sporocyst in which none of the daughters appeared ready to migrate and the largest were at about the stage shown in Fig. 14 a few 4 to 6 cell embryos were found among the germ cells. Also, in another mother 20½ days old the largest daughters contained a considerable number of embryos; in one 0.83 mm long there were 11 fairly large germ balls, the largest of which had already grown to a size of 0.061 by 0.029 mm, and a number of smaller ones.

It seemed of interest to determine how many germ cells are produced during the phase of multiplication in the daughter sporocysts. Sixteen counts of the germ cells of daughter sporocysts still within the mother gave numbers from 127 to 208 with an average of 170. These counts were difficult to make and our impression is that they are considerably short of the actual numbers. In fact, counts in three daughter sporocysts in the migrating stage which had left the mother gave 238, 227 and 236 germ cells respectively and the third had also 5 small germ balls.

Mature and old mother sporocysts, which in some cases persist long after the first daughters have escaped, vary greatly in length (from about 10 to 26 mm). Their body wall is quite thin and opaque from the increase of the minute globules in its inner layer. Such mother sporocysts when still in the snail look like inflated, elongate sausage shaped balloons, and the germinal material even before any of the daughters have escaped fills only a part of the body cavity. Their anterior ends are attenuated and birth pores and canals, as described for the mother sporocysts of *C. stagnicolae*, can be clearly seen (Cort and Olivier, 1943). Apparently the daughter sporocysts escape only from the birth pores of the mother, since empty sporocysts still intact have been not infrequently found.

#### *Time of Escape of Daughter Sporocysts and Persistence of Mothers*

As already indicated, at any given stage in the development of a mother sporocyst most of the daughter sporocyst embryos appeared to be in about the same stage of development. Thus as noted above in one mother 20½ days old, from which no daughters had yet escaped, there were numerous active daughters 0.38 to 0.83 mm in length and only eight embryos in earlier stages. This rather synchronous development of the daughter sporocyst embryos inside of the mother usually results in the escape of most of the daughters over a comparatively short period of time. The youngest infection in which we actually found daughter sporocysts outside the mother was 21½ days old. In this case 36 daughters, which were active and which appeared ready to migrate, were counted inside the mother; no earlier embryonic stages were present. The large numbers of daughters outside the mother (84 were

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FIG. 17. Optical section of a small part of a migrating daughter sporocyst from the digestive gland of a snail 22½ days after infection. Although some of the germ cells had already started to develop into cercarial embryos the germinal material was still rather tightly packed in the body cavity.

FIGS. 18 and 19. Optical sections of small portions of young daughter sporocysts from the digestive gland of snails showing development and arrangement of the germinal material.



isolated and counted) were still in the migrating stage, although the largest were about twice the length of those still inside the mother sporocyst. While this suggested that the first of these daughters probably escaped earlier than 21 days after infection, still the actual lag in development of those still within the mother was not very great. In another infection only 23 days old the mother sporocyst was entirely empty and the daughters had completed migration and had penetrated into the digestive gland. This case also indicates that the escape from the mother took place over a short period of time although it seems probable that the first sporocysts had escaped before 21 days. In another infection 27½ days old the mother was also entirely empty and the daughters had developed so far and were so interlaced in the tissues of the snail's digestive gland that it was difficult to remove any complete.

Usually, however, while most of the daughters will escape at about the same time, a few that have lagged behind the others will be found inside the mother. This is well illustrated by two infections in which the mother sporocysts 41 days after infection were still in good condition and contained a few active daughters and earlier embryos; in both cases the daughters in the tissues of the snails were almost mature and contained cercariae that appeared ready to escape.

A few of the infected snails were examined at much longer periods after infection. In 10 cases mother sporocysts containing a few daughters, most of which were active, and a few germ balls were found in snails 61 and 65 days after infection. In fact mother sporocysts with a few active daughters still in them were found in snails examined 71, 88, 126, 144 and 154 days after infection. In the group of 10 snails examined 154 days after infection, however, mother sporocysts were found in only two but in both these cases active daughters were present. Finally, in 10 snails examined 167 days after infection no mothers were found. The snails which were examined at the longer intervals after infection had been subjected to low temperatures for considerable periods of time which had delayed the development of the sporocysts. It seems clear, however, that mothers containing a few daughters can persist for a considerable time. Whether daughters that have remained in the mothers for such long periods after the others have escaped will ever migrate and develop cannot be determined, but in many old mother sporocysts, daughter sporocysts and embryos that had obviously degenerated were not infrequently found.

The general impression we have gained from the examination of large numbers of infections is that in most cases most of the daughter sporocysts of *S. douthitti* develop almost synchronously and escape from the mothers at about the same time. This produces infections in which most of the daughter sporocysts are of about the same age. In fact, in only a very few cases have we ever found immature daughters along with mature daughters that were producing cercariae.

#### *Development of Daughter Sporocysts After Leaving the Mother*

Migrating daughter sporocysts are located on the organs between the foot and digestive gland of the snails. They are very active and mobile and can extend and contract their bodies greatly. They increase considerably in size even before migration is over. In the 21½ day-old infection mentioned above, the daughters within the mother which were active and appeared ready to escape measured 0.42 to 0.74 mm, and those outside the mother which were still in the migrating stage varied from 0.78 to 2.22 mm. Due to this rapid growth the germinal material in the larger

of the migrating daughters becomes much less crowded. Also, more of the germ cells have started embryonic development, although as noted above, there is considerable variation in these respects in different infections. Fig. 16 shows a small section of a migrating daughter sporocyst 1.7 mm in length in which none of the germ cells had started development, which illustrates how the rapid growth during migration spreads the germ cells apart. On the other hand, Fig. 17 shows a section of a migrating daughter sporocyst, 1.6 mm in length, from another infection, in which some of the germ cells had developed into embryos, but in which the germinal material was more crowded. Still further spreading apart of the germinal material is shown in Fig. 18. This is drawn from a migrating daughter sporocyst which contained 236 germ cells, 5 developing embryos and one germ-mass. It is the youngest daughter sporocyst in which a germ-mass was found. This infection was started August 11, 1942, and was much retarded in development, since at 37 days after infection the mother still contained 36 daughters, and those outside the mother were very immature.

After migration most of the daughter sporocysts penetrate into the digestive gland of the snail; only a few penetrate into the tissues among the organs in front of this gland. After penetrating into the tissues they become completely immobile. Most of the germ cells soon start to develop into embryos which increase rapidly in size. The sporocysts evidently continue to increase in size, since even with the rapid development of the germinal material it fills only a part of their body cavities. The body wall of the sporocysts becomes more opaque and appears to be thinner than in the earlier stages. The wall is in very close contact with the snail's tissue and it is almost impossible to free sporocysts unbroken; in fact, the largest daughter sporocyst that was ever freed unbroken from a snail's tissue was only 2.5 mm in length, and was only slightly advanced in development beyond the migrating stage. Fig. 19 shows the posterior end of a daughter sporocyst soon after establishment in the digestive gland. As shown in this drawing the germ cells are beginning to take a position close to the inside of the wall and the largest embryos have a diameter almost equal to that of the body cavity of the sporocyst. At this stage, also, all the embryos and germ cells are still connected by strands to the wall. In slightly later stages the few germ cells that remain undeveloped are always close to the wall and more and more of the lumen is filled with the embryos. In somewhat older daughter sporocysts no undeveloped germ cells are found and the embryos are free in the body cavity, having lost their connections with the wall.

At about the stage when the largest cercarial embryos become elongate and reach a length of 0.10 to 0.15 mm, the daughter sporocysts begin to show inflated sections containing the embryonic material separated by empty constricted sections. Fig. 20 is an outline drawing of a part of a sporocyst showing the general shape at this stage. In such sporocysts undeveloped germ cells were never found. The inflated sections contain cercarial embryos in various stages of development and occasional germ-masses (Figs. 21 and 22). All attachment of the embryos to the wall has been lost. In slightly older sporocysts, in which some of the cercarial embryos are beginning to show a division into body and tail, the inflated sections become broader and are more nearly filled by the developing embryos (Figs. 23 and 24). The wall of the daughter sporocysts at this stage is quite opaque, and so thin and in such close contact with the snail's tissue that only small sections of the individual sporocysts can usually be freed.

As shown in Figs. 21, 22, 23 and 24 germ-masses similar to those found in sporocysts of *C. stagnicolae* (Cort and Olivier, 1943) may be present along with the cercarial embryos in the inflated sections of the daughter sporocysts. They are not at all common and it is unusual to find as many as three in a single inflated area. Ex-

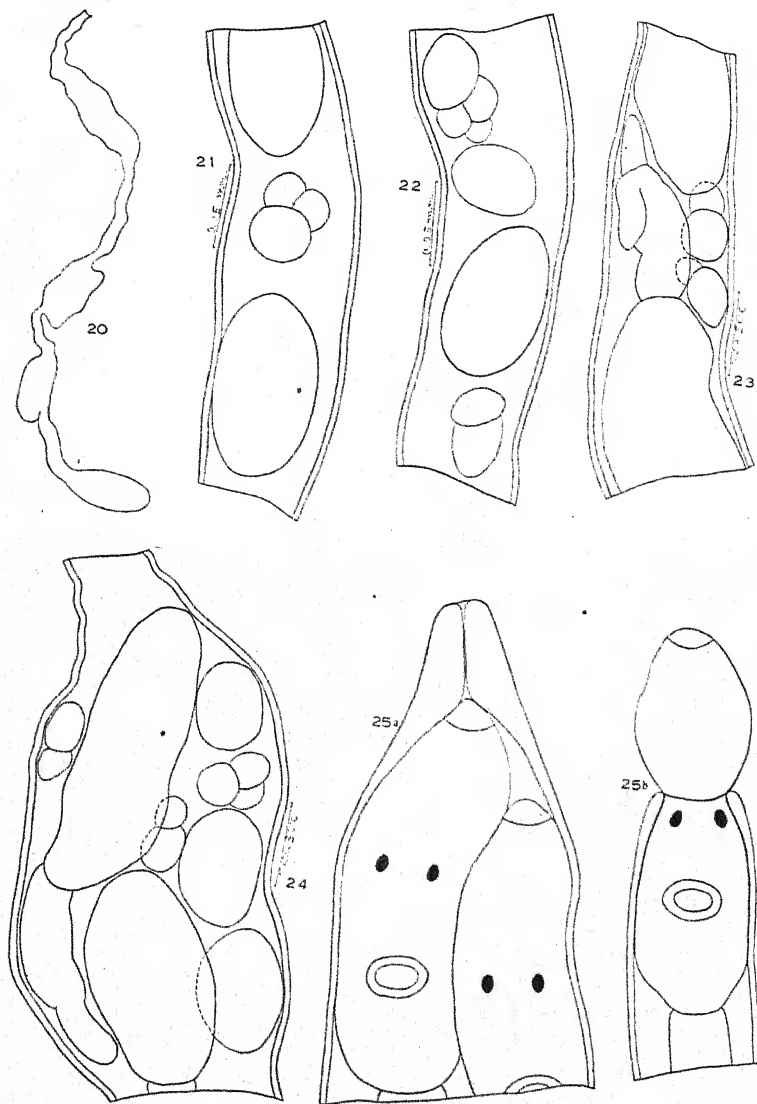


FIG. 20. Outline drawing of an incomplete daughter sporocyst from snail's tissue showing relations of the inflated sections.

FIGS. 21, 22, 23 and 24. Outline sketches showing relation of cercarial embryos and germ-masses in small sections of daughter sporocysts about the stage of figure 20.

FIG. 25a and b. Sketches showing anterior end of daughter sporocysts containing fully developed cercariae.

cept in a very few cases germ-masses were not found at stages earlier than those shown in Figs. 22 and 23; and they were very rarely present along with mature active cercariae. In some infections we were unable to find any germ-masses at all and in others numerous sections of sporocysts would be examined before any were found.

In only a few of the infections examined did we find them at all regularly and then only in part of the sporocysts and in about the numbers shown in Figs. 21 to 24. Of course, the germ-masses are difficult to find and are obscured by the opacity of the sporocyst wall, so that we could not always be sure that failure to find them was due to their absence. The germ-masses found in our experimental infections as shown in Figs. 21 to 24 seem to be almost entirely composed of multicellular components, which vary in number from two to five (usually 3 and 4). In several natural infections of sporocysts of *S. douthitti* studied at about this same stage of development small numbers of germ-masses were also found. In a few of these, unicellular components were present.

When the cercariae in an inflated section of a daughter sporocyst approach maturity they tend to crowd it to its full capacity. Often such sections appear to contain only almost fully developed cercariae. In mature infections from which cercariae are escaping the daughter sporocysts permeate the whole tissue of the digestive gland, appearing to make up at least half its bulk. The digestive gland appears to be badly injured especially in infections in which a large proportion of the sporocysts are empty and degenerate.

If the body of a snail containing a mature infection of *S. douthitti* is removed from the shell, terminal sections of the sporocysts with attenuated anterior tips and birth pores (Fig. 25a and b) will be seen extending slightly from the surface of the digestive gland. No escape of cercariae from these birth pores was ever seen until the examinations were made in the evening. To observe the escape, six snails with natural infections of *S. douthitti* were isolated in bottles and the water changed in the late afternoon. They were watched during the evening and the cercariae began to escape from them about 9:30 P.M. (Eastern War Time). At 9:45 P.M. the body of one of these snails was removed from the shell, and a number of cercariae were soon seen to emerge from the birth pores of the anterior tips of the sporocysts which projected from the surface of the digestive gland. This observation confirms the earlier findings of Cort and Talbot (1936) on the time of escape of the cercariae of *S. douthitti* from the snail host.

#### DISCUSSION

The miracidium of *S. douthitti* like those of other schistosomes contains a considerable number of germ cells in its body cavity, indicating a multiplication of the cells of the germinal line beginning at an early stage of its development. After penetration into the snail intermediate host the miracidium metamorphoses into an oval sac with these germ cells scattered throughout its body cavity (Fig. 1). As this sac grows rapidly in length the germ cells start to multiply again and come to lie close to the inside of the body wall (Figs. 2 and 3). While it would be difficult to prove, it seems probable that the phase of germ cell multiplication in the very young mother sporocyst is completed before any of them start development. In mother sporocysts about a week old the germ cells start to develop into embryos which remain attached to the wall until they have reached considerable size. All the germ cells of the mother sporocysts develop directly into daughter sporocyst embryos since no structures that had the slightest resemblance to the germ-masses that were so common in the mother sporocysts of *C. stagnicolae* (Cort and Olivier, 1943) were ever found. By the time these embryos have reached the round "germ



ball" stage they consist of a mass of germ cells enclosed in a thin cellular membrane. As these embryos elongate the wall becomes thicker and the enclosed germ cells increase in number. They can be seen to have cytoplasmic processes connecting them with the inside of the body wall and with each other. With further increase in length these embryos develop into elongate motile daughter sporocysts with a well differentiated body wall, which contain a large number of germ cells. Either just before or just after the daughters escape from the birth pore of the mother their germ cells start to develop into embryos. After the daughter sporocysts leave the mother they migrate along the organs of the snail and penetrate into the tissues chiefly in the digestive gland. After penetration they become immobile and later appear as much larger irregular elongate sacs with inflated sections containing the developing embryos and sometimes a few germ-masses separated by narrow empty sections. The attenuated anterior ends of the daughter sporocysts with birth pores extend slightly from the surface of the digestive gland of the snail.

In the experimental infections started in *S. p. clodes* about the first of August, 1942, the first daughter sporocysts escaped from the mother about three weeks after infection and the first cercariae began to escape from the daughters at about 6 weeks. It, therefore, took about two weeks for the germ cells of the mother to develop into migrating daughters and about three weeks for the germ cells of the daughters to become active cercariae. With warmer temperatures the time of development would undoubtedly be considerably shorter. Also, in the experimental infections started later in August development was much slowed up by cooler weather.

Multiplication of individuals in the sporocyst stages of *S. douthitti* is brought about almost entirely by two phases of multiplication of the cells of the germinal line. One of these starts in the early embryo of the miracidium and is completed several days after the mother sporocyst has started its development in the intermediate host. This results in the production of a considerable number of daughter sporocysts (from about 200 to over 400) inside the mother, and each germ cell of the mother appears to develop directly into a daughter. The second phase of germ cell multiplication must start very early in the development of the daughter sporocyst embryos because in germ balls less than 0.05 mm in diameter the soma is represented only by a thin membrane in which only a few nuclei can be seen, and a number of germ cells are already present. As the outer membrane differentiates to form the wall of the daughter sporocysts the germ cells increase in number to about 200 before they start embryonic development.

It is evident that all but a few of these germ cells in the daughter sporocyst embryos develop directly into cercarial embryos. A very few of them, however, must develop into the germ masses which are found in small numbers in some of the developing daughter sporocysts. These, we suggest, represent a very brief phase of polyembryony in which a single germ cell divides and its divisions start development without separating. When these multicellular components of the germ-mass reach a certain size they break apart and each develops into a cercaria. Thus in the daughter sporocysts of *S. douthitti* it would appear that each of the few germ cells that develop into germ-masses finally produces several cercariae. Since these germ-masses are entirely absent in the mothers and so few in number in the daughters, it is evident that polyembryony of germ-masses in this species plays only a very minor part in the multiplication of individuals. A conservative estimate would be

that in *S. douthitti* from the fertilized ovum that produces the miracidium 40,000 to 60,000 cercariae are produced, almost entirely by the two phases of direct multiplication of germ cells. The multiplication of individuals in the germ cell cycle in this species, therefore, consists of four phases, viz., (1) the production of large numbers of fertilized ova by the adults, (2) multiplication of cells of the germinal line in the miracidium-mother sporocyst stage, (3) multiplication of cells of the germinal line in the daughter sporocyst stage, and (4) a very brief and irregular phase of polyembryony in the daughter sporocyst stage.

Since in an earlier paper (Cort and Olivier, 1943) on the embryology of the sporocysts of *C. stagnicolae* the meagre information in the literature on the development of the sporocysts of the schistosomes was discussed, it will be sufficient to point out here the differences between *C. stagnicolae* and *S. douthitti*. In general appearance and structure the mother sporocysts of these two species are very similar although their location in the intermediate host is entirely different. Also, in the early stages of development the germinal material in the mother sporocysts of *C. stagnicolae* does not line the wall but is suspended by strands in the lumen. A fundamental difference between these two species is the presence in the mother sporocysts of *C. stagnicolae* of a considerable number of germ-masses which are completely absent in this stage of *S. douthitti*. So polyembryony in the mother sporocysts of *C. stagnicolae* appears to be an important method of increasing the number of daughter sporocysts, while it is entirely absent at this stage in *S. douthitti*. There is, therefore, a greater lag in the production of daughter sporocysts in the former species and a tendency for their development and escape to spread over a longer period of time.

The structure of the stages of development of the daughter sporocysts of the two species inside the mother is very similar. In these stages of *C. stagnicolae* the multiplication of germ cells seems to last longer, since when the daughters in this species escape from the mother the germ cells in their body cavity are very crowded and none was ever seen that had started development. In fact, in this species we never saw cytoplasmic projections from the germ cells of daughters while still within the mother, although judging from later stages they must be present. It can be suggested that the extent of crowding of the germ cells in the developing daughter sporocysts depends on the time relations of germ cell multiplication to growth in size. In *S. douthitti* there is considerable variation in this respect. Apparently in some cases the multiplication of the germ cells inside the daughter sporocyst embryos continues until the daughters are ready to escape. This results in a condition like that of *C. stagnicolae* in which the germ cells are rather crowded and none has started embryonic development. In a few cases in *S. douthitti*, however, it appears that the germ cell multiplication in the daughters inside the mother is completed earlier, resulting in the spreading apart of the germ cells and some precocious development of embryos in the daughter sporocysts before they escape from the mother.

Another difference between these two species is the presence in *C. stagnicolae* of large numbers of germ-masses in the early stages of the development of the daughter sporocysts outside the mother. In fact, these structures are so common that the suggestion was made that possibly all the germ cells go through this stage before producing cercarial embryos. Whether this is true or not, it is evident that polyembryony of germ-masses in the daughter sporocysts of *C. stagnicolae* is a very

important factor in the multiplication of individuals while in *S. douthitti* it seems to be a very minor factor, and perhaps only occasionally present.

While actual counts are not available we have a clear impression that in *C. stagnicolae* cercarial production is very much greater than in *S. douthitti*. Also, cercarial production in the former species appears to extend over a considerably longer period than in the latter. The extent of polyembryony of germ-masses in the former species and its almost complete suppression in the latter would account for these differences. In fact, it seems possible that *S. douthitti* is exceptional among schistosome species in the small number of cercariae produced and in the comparatively short period in which they escape from the snail host. We certainly have the impression that cercarial production is also very much greater in the other two schistosome cercariae of the Douglas Lake region, *C. elvae* and *C. physellae*. It is of special interest in this connection that in a limited series of observations we were able to make on immature mother sporocysts of *C. elvae* in natural infections we found germ-masses with about the same frequency as in *C. stagnicolae*. We know of no records of the presence of germ-masses in the sporocysts of other schistosome species. It is of interest, however, that studies of single miracidium infections in *Schistosoma mansoni* (Faust and Hoffman, 1934) indicated a considerably greater cercarial production over a very much longer period than for *S. douthitti*. In one such infection 210,000 cercariae were actually counted and the snail was still discharging more than 2,500 cercariae per day when the counts were discontinued. It can be suggested, therefore, at least as a working hypothesis, that the presence of a considerable number of germ-masses with a significant polyembryony is typical of the schistosomes, and that the situation in *S. douthitti* is unusual for the group. However this may be, it seems evident that in the germ cell cycle of the schistosomes the direct multiplication of the cells of the germinal line is very important in the production of large numbers of Cercariae, and that polyembryony of germ-masses which in the strigeids accounts for most of the multiplication, is relatively of less significance.

#### SUMMARY

In the summers of 1941 and 1942 studies were made on the development of the mother and daughter sporocysts of *Schistosomatium douthitti* in experimentally infected snails. In the youngest mother sporocysts only germ cells were present, which increased in numbers to about 200. These germ cells developed directly into daughter sporocysts, which escaped from the birth pore of the mother after about three weeks. Germ cells localized in the body cavities of the daughter sporocysts increase in numbers to about 200 when the daughters are ready to escape from the mothers. Most of these develop directly into cercarial embryos, the first of which mature in about six weeks. A few germ-masses are present in the daughter sporocysts after they are established in the snails' tissues, suggesting that a brief phase of multiplication by polyembryony is also involved. It was estimated that from 40,000 to 60,000 cercariae are produced from one fertilized ovum, almost entirely by direct multiplication of the cells of the germinal line in the mother and daughter sporocysts. The almost complete elimination of multiplication by germ-masses in the sporocysts of this species appears to have limited cercariae production, as compared with species like *C. stagnicolae* and *C. elvae* where these structures are numerous in both the mother and daughter sporocysts. It is evident that direct multiplication of



the cells of the germinal line in the mother and daughter sporocysts plays a much greater role in the multiplication of individuals in the schistosomes than in the strigeids, since in this group multiplication is chiefly by polyembryony of germ-masses.

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# THE EFFECT OF CONCURRENT INFECTION WITH *NIPPOSTRONGYLUS* ON THE DEVELOPMENT OF *HYMENOLEPIS* IN MICE<sup>1</sup>

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A few observations have been made but little or no specific work has been done to show what effect the presence of one species of parasite has on the development of a second. Brumpt (1933) noticed that the resistance of one mouse to reinfection with *Hymenolepis nana* var. *fraterna* was broken down by a secondary infection with *Strongyloides*. Apparently there are no reported observations on the mutual effect of two different parasites following simultaneous entry into a host. It was to obtain such information that the present study was undertaken, since the nematode, *Nippostrongylus muris*, and the cestode, *H. nana* var. *fraterna*, were both available in the laboratory at the same time.

## METHODS

Quantitative doses of *Hymenolepis* eggs isolated from host feces which had been stored 48-72 hours were administered to mice by stomach tube, and 93-hour cysticercoid counts were made after preparing the intestine free from mucus (Larsh, 1942). Infective *Nippostrongylus* larvae were isolated from 10-20 day old cultures, washed by centrifugation in normal saline, and counts made by a modification of Scott's (1928) dilution count. Known numbers of larvae were injected subcutaneously into mice in the abdominal wall just medial to the flank.

## EXPERIMENTAL DATA

Three preliminary experiments were performed in which a total of 24 mice, 2-3 months old, were used. Half of these were infected subcutaneously with 1000-1500 *Nippostrongylus* larvae per mouse just prior to receiving 1200-1350 eggs of *Hymenolepis* per mouse. The other 12 mice which were used as controls received only the tapeworm eggs. In the first experiment, the four experimental mice had an average percentage development of cysticercoids of 1.3, and the controls 2.2. The second experiment showed a striking difference between the groups as the four experimentals averaged 1.6, and the controls 4.5. The third experiment was more nearly comparable to the first in that the experimentals averaged 1.2, and the controls 2.7. No record was kept of the *Nippostrongylus* infections, since, at this time, the larvae are still in the migratory stage. These results were striking enough to suggest an interesting relationship worthy of further study, so that two other experiments were performed in which larger numbers of animals were involved and the doses of larvae and eggs kept constant.

In the first experiment, twenty mice two months old were selected. Ten of these were each infected subcutaneously with 2000 larvae of *Nippostrongylus* and within

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an hour were each given 4200 *Hymenolepis* eggs. The ten controls were given a corresponding number of eggs of *Hymenolepis* from the same culture. In the second experiment, another twenty mice, two months old, were used and the ten experimental animals each received 2400 larvae of *Nippostrongylus*. Later, along with the controls, they were each given 2150 eggs of *Hymenolepis*. The results of the two experiments are shown in Table 1.

An examination of the table shows that the two experiments are markedly similar and that the number of cysticeroids of *Hymenolepis* in the controls was about twice the number observed in the experimental group. In the first experiment, the controls had a percentage development of cysticeroids of *Hymenolepis* of 6.0, compared with 3.7 in the mice infected with both parasites, and, in the second experiment, the controls averaged 6.6, the experimentals 3.0 per cent. These results agree, therefore, with those of the three preliminary experiments, so that in all a total of 32 experimental and 32 control mice were observed.

TABLE 1.—Showing the percentage development of cysticeroids in mice infected with both *Hymenolepis* and *Nippostrongylus* and in mice infected only with *Hymenolepis*

Exper. no.	No. of mice	Infesting egg dose per mouse	Ninety-three hour cysticeroids		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. Mice infected simultaneously with <i>Hymenolepis</i> and <i>Nippostrongylus</i>					
1	10	4200	156.3	19.2	3.7
2	10	2150	64.6	18.0	3.0
B. Control mice infected only with <i>Hymenolepis</i>					
1	10	4200	253.5	32.3	6.0
2	10	2150	142.4	20.9	6.6

The data of the last two experiments, Table 1, were analyzed statistically. In the first experiment, the observed difference between the percentage development of the cysticeroids in the experimental and control groups was 7.7, and in the second experiment 8.4, times the standard deviation. Grouped together for analysis, the two experiments show an even more pronounced significance, since the observed difference between the percentage development of the total animals of the two groups was 10.9 times the standard deviation. Hence it is extremely unlikely that the differences shown in the table could have occurred by chance.

The results of these experiments indicate that the infections with *Nippostrongylus muris* in some way or other reduced the numbers of the cysticeroids of *H. nana* var. *fraterna* that were able to develop.

#### SUMMARY

Young white mice infected with *Nippostrongylus muris* just prior to infection with *H. nana* var. *fraterna* exhibited a marked resistance to the tapeworm and harbored only one-half the number of cysticeroids observed in controls of the same

age infected only with the cestode. No suggestion is offered to explain this unusual phenomenon, but further work is planned to study the mechanism involved.

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# COMPARATIVE STUDIES ON A MOUSE STRAIN OF *HYMENOLEPIS NANA* VAR. *FRATERNAL*, IN DIFFERENT SPECIES AND VARIETIES OF MICE<sup>1</sup>

JOHN E. LARSH, JR.<sup>2</sup>

As a result of the many studies on *Hymenolepis nana* var. *fraterna* in white mice, much information has been presented on the relations between this parasite and host. Few data have been published, however, on any of these relations in other species and varieties of mice. Hunninen (1935) compared the percentage development of the parasite in white mice with that in deer mice (*Peromyscus maniculatus*) and other rodents, but he had opportunity to test only a small number of the wild mice. Therefore, when the writer was presented with several mating pairs of deer mice and house mice (*Mus musculus*), it seemed worth while to make further tests with these hosts. In addition, a series of other comparisons were made between white and dilute brown laboratory mice.

## EXPERIMENTAL DATA

The first comparison between the albino and wild mice was in the number and location of cysticercoids after initial infections (Table 1). The first point of interest in table 1 is the presence in all three groups of about the same percentage development, the range for the white mice, house mice, and deer mice being respectively

TABLE 1.—A comparison of the percentage development and location of cysticercoids of *H. nana* var. *fraterna* in white mice, *M. musculus* and *P. maniculatus*

No. of mice	Age of mice (days)	No. eggs per mouse	Ninety-three hour cysticeroids				
			Average no.	Standard deviation of the individual measurements	Per cent in first half of intestine	Per cent in second half of intestine	Percentage development
A. White Laboratory Mice							
5	51	2700	98	6.8	85	15	3.5
4	25	2400	71.5	8.5	76	24	2.9
4	30	2000	63	6.4	94	6	3.0
4	60	2400	93.7	6.5	88	12	3.8
B. <i>M. musculus</i>							
5	51	2700	92.4	10.4	81	19	3.3
4	25	2400	67.2	8.9	84	16	2.7
4	30	2000	41.7	9.2	91	9	2.0
4	180	2400	87.2	9.5	92	8	3.6
C. <i>P. maniculatus</i>							
5	51	2700	85.6	5.1	34	66	3.1
4	25	2400	89.7	8.6	26	74	3.9
4	30	2000	90	14.6	25	75	4.4
4	180	2400	91	14.7	39	61	3.7

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2.9–3.8, 2.0–3.6, and 3.1–4.4. The second point of interest is the lack of resistance exhibited by the old (180 days) house mice and deer mice which had about the same percentage development as the younger mice. This is in contrast to the marked resistance shown by old white mice of this age (Shorb, 1933, Hunninen, 1935). Unfortunately, the three hosts could not be compared directly because no white mice 180 days old were available at the time of the experiment. A third point of interest is the location of the cysticercoids in mice of the three groups. The number found in the first half of the small intestine of the white mice and house mice was 76 per cent or more of the total number which agrees with Hunninen's (l.c.) figures for white mice. However, in the deer mice the reverse was true and only 25 to 39 per cent were observed in the corresponding region while most of the others were located in the posterior one-fourth of the small intestine. This unusual location of the cysticercoids was found in all the deer mice including those 180 days old.

The next experiments were performed to make a comparison between the different kinds of mice in the number and size of adult worms found after test infections; in all cases, the mice were autopsied 11 days following infection. The number of worms developing in the white and house mice was about the same, but very few were found in the deer mice (Table 2). The range in percentage development of

TABLE 2.—A comparison of the number of 11-day worms of *H. nana* var. *fraterna* in white mice, *M. musculus* and *P. maniculatus*

No. mice	Age of mice (months)	Egg dose per mouse	11-day adult worms		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. White laboratory mice					
5	2½	2000	37.2	11.9	1.9
3	3½	2500	58.6	8.6	2.3
3	5½	2500	62.6	15.5	2.5
B. <i>M. musculus</i>					
5	2½	2000	30.8	10.0	1.5
3	3½	2500	63.3	5.0	2.5
3	5½	2500	60.0	14.7	2.4
C. <i>P. maniculatus</i>					
5	2½	2000	1.00	1.260	0.05
3	3½	2500	0.66	0.027	0.03
3	5½	2500	0.00	0.000	0.00

the worms in the white mice was 1.9–2.5, in the house mice 1.5–2.5, and in the deer mice 0.03–0.05. Another point brought out is that the old house mice (5½ months) had about the same percentage development of the adult worms as the younger mice. Those tested when 2½ and 3½ months old had a percentage development of 1.5 and 2.5, respectively, compared with 2.4 at 5½ months. A third interesting point is the differences in the size of the worms from the three hosts. Those recovered from the house mice were always larger than those from the white mice while the few worms found in the deer mice were very much smaller. In the first series when 2000 eggs were fed, the average size of the 186 worms from the five white mice was 39 mm. (range: 30–51), that of the 154 worms from the house mice was 49 mm. (range: 38–67), and the average size of the 5 worms from the deer mice was 3 mm.

(range: 2-8). The relationship in size was similar in the other two experiments, but, as stated above, direct comparisons were not possible because white mice of the same age as the wild mice were not available when needed. It was found that size is not necessarily associated with host age as the worms recovered from the old house mice (5½ months) were about the same size as those from the younger mice of this species.

In the comparisons between the white and dilute brown<sup>3</sup> mice, the first point to settle was whether the two hosts differ in their natural susceptibility to *H. nana* var. *fraterna*. This determination was based on the results of five experiments in which a comparison was made of the number of 93-hour cysticercoids present after test infections of stored eggs (Larsh, 1943b). In every case, the white mice, from 52-82 days old, harbored considerably more parasites than the brown mice of the same age given similar infections from the same egg culture (Table 3). The white

TABLE 3.—Showing the percentage development of cysticercoids in white and dilute brown mice infected with different egg doses of *H. nana* var. *fraterna*

Exper. no.	No. of mice	Age of mice (days)	Egg dose per mouse	Ninety-three hour cysticeroids		
				Average no.	Standard deviation of the individual measurements	Percentage development
A. White Laboratory Mice						
1	8	52	900	36.5	7.0	4.0
2	4	75	1200	54.0	8.3	4.5
3	3	82	1500	61.6	5.3	4.0
4	5	75	2000	101.8	9.5	5.4
5	5	75	2000	112.4	7.5	5.6
B. Brown Laboratory Mice						
1	8	52	900	5.3	3.9	0.6
2	4	75	1200	23.5	8.6	1.9
3	3	82	1500	37.0	4.0	2.4
4	5	75	2000	54.2	13.5	2.7
5	5	75	2000	47.0	9.7	2.4

mice showed a range in percentage development of cysticercoids from 4.0 (first and third experiments) to 5.6 (fifth experiment), while the range for the brown mice was from 0.6 in the first to 2.7 in the fourth experiment.

The next tests were designed to obtain data on the adult stage of the parasite, all the mice being killed eleven days after infection when the number and size of the worms were recorded. In the three experiments performed, each of the 18 white mice harbored more than twice as many worms as the brown mice (Table 4). The range in percentage development was from 2.3 to 3.2 in the white mice, and from 1.0 to 1.9 in the brown mice. The worms recovered from the two hosts did not vary greatly in size. Those from the white mice after an infection of 3500 eggs, while showing the usual variations (10-61 mm.), averaged 31.5 mm. in length, as compared with 26.2 mm. in the brown mice where the range in size was 6-43 mm.

The last tests were performed to determine the prepatent and patent periods of the worms in the two hosts. Twelve mice, 2½ months old, of each variety were infected with 1500 eggs per mouse. After the tenth day, all the fecal pellets collected over a 12-hour period each day were examined for eggs by the Willis salt-floitation method. Five white mice began passing eggs on the fifteenth day, four on

<sup>3</sup> The C57 strain bred in the Jackson Memorial Laboratory, Bar Harbor, Maine.



TABLE 4.—Showing the percentage development of adult *H. nana* var. *fraterna* in 2½-month-old white and dilute brown mice infected with different egg doses

Exper. no.	No. of mice	Egg dose per mouse	11-day adult worms		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. White Laboratory Mice					
1	5	2000	52.4	13.6	2.6
2	8	2000	45.0	12.0	2.3
3	5	3500	113.2	12.6	3.2
B. Brown Laboratory Mice					
1	5	2000	20.8	7.6	1.0
2	8	2000	23.1	8.7	1.1
3	5	3500	67.2	13.1	1.9

the seventeenth, two on the eighteenth, and one on the twenty-second day. Five of the brown mice began passing eggs on the eleventh day, six on the twelfth, and one on the thirteenth day. The length of the patent period was also recorded by using these same twelve mice of each variety and later by adding an additional six in each group which were infected with the same number of eggs, i.e., 1500. Daily fecal examinations were continued until each animal had been negative for seven days, then the absence of worms was verified by autopsy. The patent period of the worms in the white mice ranged from 20 to 48 days, and in the brown mice from 16 to 30 days.

#### DISCUSSION

After test infections of the same size, similar numbers of cysticercoids of *H. nana* var. *fraterna* were observed in white mice, deer mice, (*Peromyscus maniculatus*) and house mice, (*Mus musculus*), but in later experiments, the deer mice harbored only a few adult worms while the other two hosts had equally large numbers. These results, in general, are in harmony with those of Hunninen (1935) who tested white mice, deer mice, rats and guinea pigs and found a somewhat similar development of cysticercoids in all hosts; but, in other experiments, he observed very few adults in any but the white mice. He pointed out that the experiment showed a specificity of the adults and a lack of specificity of the cysticercoids. This same relationship was demonstrated again in the above series of experiments. It is interesting that the old house mice (180 days) harbored about the same number of cysticercoids and adults as the young mice of this species because old white mice are resistant to infection. If more extensive study proves that old house mice are as susceptible in all cases, then it may offer some support to Sandground's (1929) hypothesis that age immunity is merely a type of natural immunity. This author contends that age immunity is shown typically by a host against only abnormal, newly acquired, or imperfectly adjusted species of parasites. Thus the reason old white mice exhibit strong resistance to *Hymenolepis* may be due to slight physiological changes produced in establishing the strain from the wild *Mus musculus*. Regardless of the view taken, *Mus musculus* appears to be a better host for this tapeworm than white mice heretofore referred to as the "normal" host.

It is clear from the above data that certain differences exist between the white and the brown laboratory mouse in their relationships with *H. nana* var. *fraterna*.

The smaller numbers of cysticeroids and adult worms in the brown mice and the shorter patent period would seem to suggest that they are less favorable for the development of the parasite than white mice. It is, therefore, rather surprising that the parasite has a shorter prepatent period in the brown mice, because this indicates more rapid development to sexual maturity. The difference in intestinal length in these two varieties of mice might be suggested as a possible explanation for the difference in the number of worms that develop in them. A series of measurements for mice 75–82 days old showed that the intestinal length of the brown mice averaged 35.5, while in the white mice it was 45 cms. In an earlier paper (Larsh, 1943a), experimental evidence was presented in support of the view that the smaller intestinal size of young white mice is an important factor in their lower susceptibility to *Hymenolepis* than mice 2–3 months old. No test was made of this relationship in the above experiments, but the suggestion is presented as one possibility for the reduced susceptibility of the brown mice because the size of their intestine is similar to that of the young white mice studied previously. However, whatever explanation is presented to account for the results of the study must give consideration to the very apparent factor of genetic constitution which differs in the two hosts.

Ackert and his coworkers (1935) were the first to give evidence of different degrees of host susceptibility to helminthic infections between breeds of the same species. They studied chickens infected with the nematode, *Ascaridia lineata*, and found that the heavy breeds, e.g., Rhode Island Reds, were the least susceptible while the lighter breeds, e.g., White Leghorns, were the most susceptible.

#### SUMMARY

The host relations of *H. nana* var. *fraterna* were tested in different species and varieties of mice. After the same test infections, white mice, house mice (*Mus musculus*), and deer mice (*Peromyscus maniculatus*) showed about the same number of cysticeroids, but in later tests the deer mice harbored only a few adult worms whereas similar large numbers were recovered from the other two hosts. *Mus musculus* appears to be the best host for this parasite, because the worms grow to a larger size than in other hosts tested, and old mice of this species are as susceptible as younger members. Comparisons between white and dilute brown mice showed that after the same test infections, the brown mice harbored fewer cysticeroids and adults than did white mice of the same age. The adult worms in the two hosts grew to about the same size, but the prepatent and patent periods were shorter in the brown mice.

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EFFECT OF LONG ULTRAVIOLET AND NEAR VISIBLE RADIATION  
ON THE EGGS OF THE NEMATODES *ENTEROBIUS VER-*  
*MICULARIS* AND *ASCARIS LUMBRICOIDES*

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The effect on nematode eggs of ultraviolet radiation shorter than 3000A has been reported in a number of publications. The present authors (Hollaender, Jones, and Jacobs, 1940) have reported on the effect on eggs of *Enterobius vermicularis* of radiation with wavelengths between 2180 and 3000A, the greatest sensitivity of the eggs to radiation being noted at wavelengths below 2400A. The effects of sunlight on nematode eggs, especially eggs of the various species of *Ascaris* also have been under consideration for a number of years. Under the conditions of many of these experiments with sunlight there existed very little chance that the eggs received ultraviolet shorter than 3000A in sufficient quantity to produce toxic effects, but in spite of the practical absence of the shorter ultraviolet, definite inactivating effects have been observed.

By recently available experimental arrangements, it has become possible to produce high intensity radiation in the range of 3500A to 4900A, i.e., in the long ultraviolet and near visible range. Effects of these high intensities are comparable to those produced by sunlight in which the shorter ultraviolet has been excluded. This report is concerned with the results observed in using radiation of the 3500 to 4900A range on eggs of *Enterobius vermicularis* and *Ascaris lumbricoides*.

MATERIAL AND METHODS

*Source of radiation.*—As a light source there was used a water-cooled high-pressure quartz capillary mercury vapor lamp of the Daniels-Heidt type (150 to 250 volts and 2 to 4.5 amperes). Radiation from the capillary lamp was concentrated by means of a quartz condensing lens and passed through a liter round-bottom glass flask filled with a dilute solution of copper sulphate. The light then passed two Corning glass filters #585—3 mm and #738—2 mm. The beam was deflected by means of a right angle reflection prism to a platform on which was placed a uranium glass plate. The light beam covered an area of 1.5 by 5 cm clearly visible on the fluorescent plate. Usually two or three dishes of eggs were placed in the beam, and their location interchanged every 10 to 20 minutes, depending on the time of exposure. The arrangement of the equipment is shown in Fig. 1.

The intensity of the beam was measured by means of a standardized thermopile-galvanometer arrangement. The intensities obtained varied between 10,000 and 300,000 ergs per cm<sup>2</sup> per second. Spectrograms taken of the radiation coming to the ova covered the range between 3500 and 4900A. The most intense lines were the 3650A set followed by the 4358 group and finally by the 4046 set and some minor lines. Only very little radiation longer than 4358 was present. Practically all infra red radiation was absorbed by the CuSO<sub>4</sub> solution and no measurable quantity of ultraviolet shorter than 3500A could be detected.

*Eggs of Enterobius vermicularis.*—Eggs of *E. vermicularis* were irradiated in the infective stage. As in other experimental work with this parasite by the present authors (1940), eggs were obtained from female worms which had been collected while migrating; the eggs were incubated at 37° C for about 7 hours for development of the vermiform infective-stage embryo, then stored in a refrigerator until used. With one exception, eggs were used within a week after incubation. Only eggs from one worm were used in each experiment. During irradiation the eggs either were floating on water in small dishes, or were kept dry, adhering somewhat to the bottoms of the dishes. With few exceptions each sample consisted of 150 to 200 eggs. The criterion of effect of irradiation was the subsequent ability of living larvae to hatch in artificial digestive juice; this four-hour hatching test was begun immediately following irradiation.

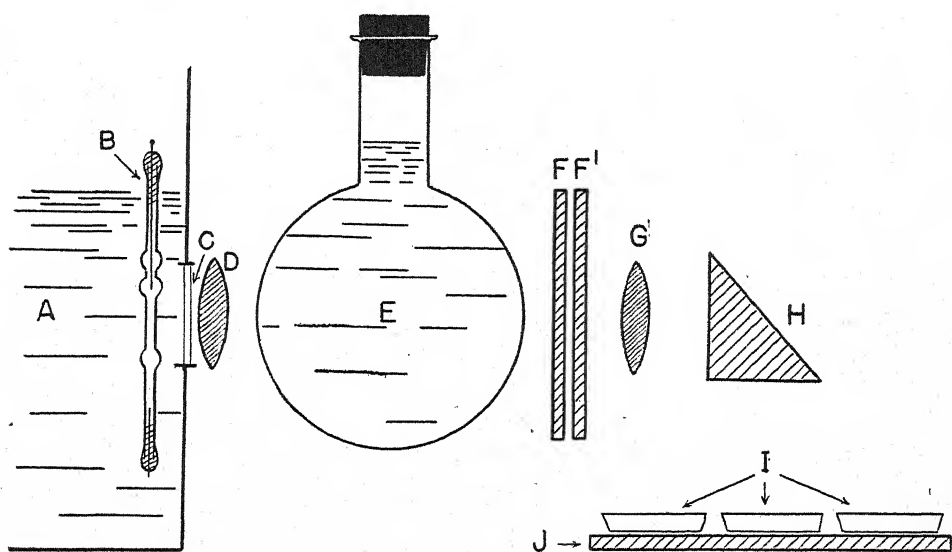


FIG. 1. Apparatus. A, Water bath for radiation source. B, Capillary mercury vapor lamp. C, Quartz window of lamp tank. D, Quartz condensing lens. E, Pyrex glass bottle with  $\text{CuSO}_4$  water solution. F and F', Corning glass filters #738 and #585. G, Quartz condensing lens. H, Right angle reflection prism. I, Dishes containing eggs. J, Fluorescence plate.

To obtain information regarding temperature factors, in certain experiments the dishes of eggs were kept on a warm plate, and in others were surrounded by cracked ice. However, eggs were not subjected to conditions of temperature or humidity which would, *per se*, noticeably decrease their viability during the time of exposure to radiation, which did not exceed 4 hours.

*Eggs of Ascaris lumbricoides.*—Eggs were obtained from worms which had been passed by patients after treatment. They were stored in shallow dishes of water in a refrigerator until used. Certain of the eggs were incubated at 30° C to obtain late-segmentation stages and embryonated forms. Most of the eggs, however, were in the one-cell stage when irradiated. During irradiation, the eggs were in shallow dishes, either dry or in distilled water. Effort was made to have eggs in single layers so that all eggs were similarly exposed. In some experiments, samples of eggs for testing were taken from a dish at intervals; in other experiments, eggs were in separate dishes which were removed at appropriate times. Some of the embry-



onated eggs were on small glass coverslips which could be mounted on a slide to facilitate observation after irradiation.

The criterion of effect of irradiation on eggs in the one-cell and the late-segmentation stages was the subsequent ability to complete embryonation at 30° C. Embryonated eggs were checked for activity of the vermiform embryo when warmed to approximately 37° C. The last type of test was less satisfactory quantitatively and resulting data are not considered as reliable as those for the tests based on development. However, after irradiation with the higher energy values, visible damage to the embryo was evident.

In earlier experiments it was not possible to hold eggs longer than 15 days after irradiation, but in later tests eggs were kept as long as 28 or 30 days for final observation.

All eggs were irradiated at room temperature which varied from 24° to 29° C; no data were obtained concerning possible temperature factors.

#### RESULTS

*E. vermicularis*.—Data of experiments with *E. vermicularis* are summarized in Table 1. Considerable inconsistency is noted between experiments at room temperatures (between 20° and 30° C) in which the maximum exposure was to radi-

TABLE 1.—Summary of data on effect of irradiation with 3500 Å to 4900 Å on eggs of *E. vermicularis*

Exp. No.	Date	Age of eggs (days)	Eggs dry or wet	Temp. °C	Relative humidity (per cent)	Minimum exposure $\times 10^7$ ergs per $\text{cm}^2$	Survival	Maximum exposure $\times 10^7$ ergs per $\text{cm}^2$	Survival
1	8/13	5.5	dry	30–31.5	65	50.2	+	200.	0
2	8/14	6.5	wet	30	..	7.5	+	24.5	+
3	8/15	7.5	wet	28–29	..	18.8	+	24.5	+
		7.5	dry	28–29	50	18.8	+	24.5	0
4	10/2	5.5	wet	21.5–24.5	..	..	..	6.3	+
		5.5	dry	21.5–24.5	48	..	..	6.3	+
5	10/8	3.5	wet	27–29	..	15.7	+	23.6	+
		3.5	dry	27–29	50	15.7	+	23.6	+
6	10/9	4.5	wet	28	..	89.	+	145.	0
		4.5	dry	28	50	..	..	145.	0
7	10/10	5.5	wet	25	..	17.	+	26.8	+
		5.5	wet	25	..	13.1	+	26.8	+
		5.5	wet	37–39	..	13.1	+	26.8	+
8	12/11	3.5	wet	23–25	..	33.	+	134.	+
		3.5	wet	35–38	..	67.	+	134.	+
9	12/19	(a) 1.5 <sup>a</sup>	wet	37	..	40.	+	100.	0
10	12/20	(a) 2.5	wet	8–13	..	35.	+	89.	+
		(b) 2.5	wet	8–13	..	35.	+	125.5	+
11	12/23	(a) 5.5	wet	8–15	..	74.7	+	199.2	+
		(b) 5.5	wet	8–15	..	87.2	+	199.2	+
12		(a) 5.5	wet	23–28	..	74.7	+	149.6	0
		(b) 5.5	wet	23–28	..	49.8	+	149.6	0

<sup>1</sup> Less than 10% hatching.

<sup>2</sup> Damaged larvae.

<sup>a</sup> a and b represent eggs from 2 different worms.

ation less than  $50 \times 10^7$  ergs per  $\text{cm}^2$ . In some tests there was considerable killing effect by low energy values of about  $25 \times 10^7$  ergs per  $\text{cm}^2$ ; however, whether these results were correlated with less resistant material, unmeasured fluctuation in light source, or other factors, it was apparent that such radiation could not be relied upon to kill eggs under all circumstances. It was only with much greater energy values that consistent killing at room temperatures was observed.

Figure 2 presents data from experiments designed to test the importance of a temperature factor correlated with the effect of radiation. In each test, eggs of the

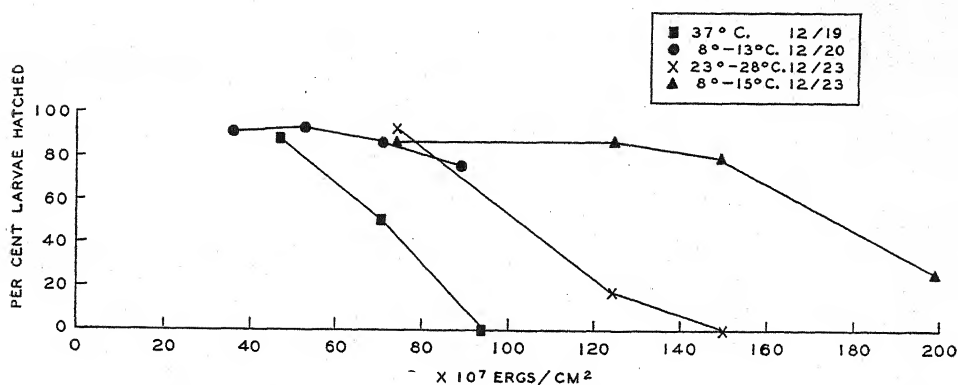


FIG. 2. Effect of 3500 to 4900 Å radiation on eggs of *Enterobius vermicularis* exposed at three different temperature ranges.

same lot were used, with the same lamps. In some cases it was possible to irradiate eggs at different temperatures on the same day; if not, exposures were made at the higher, possibly more effective temperature on the first day. Consistent results were observed. At temperatures between 35° and 38° C the effect of irradiation was most pronounced; at temperatures between 23° and 28° C killing was less rapid. At temperatures below 20° C, that is between 8° and 15° C, many eggs survived exposures up to  $199 \times 10^7$  ergs per cm<sup>2</sup>. It was noted, however, that although many larvae were able to hatch after such exposures, some showed evidence of damage, such as a brownish color or sluggish movements. It is probable that continued irradiation could have been effective, even at these low temperatures.

Comparisons of effect of radiation on dry eggs and eggs on water indicate that there was little difference in the tests with approximately 75 per cent of wet eggs hatching after irradiation. In the few tests which indicated some difference, dry eggs were apparently the less resistant.

*Ascaris lumbricoides*.—Results of the first series of experiments with eggs in the one-cell stage were checked on the basis of embryonation at 12 to 15 days after irradiation. Exposure of less than  $30 \times 10^7$  ergs per cm<sup>2</sup> produced slight or no effect. Radiation of 60 and  $80 \times 10^7$  ergs per cm<sup>2</sup> resulted in embryonation of only

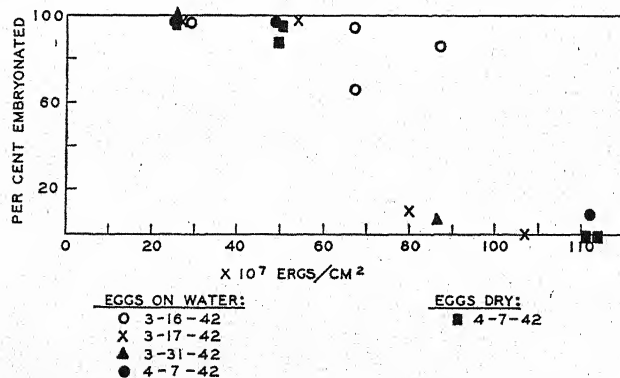


FIG. 3. Effect of 3500 to 4900 Å radiation on one-celled eggs of *Ascaris lumbricoides*. Final check on development after 4 weeks at 30° C.

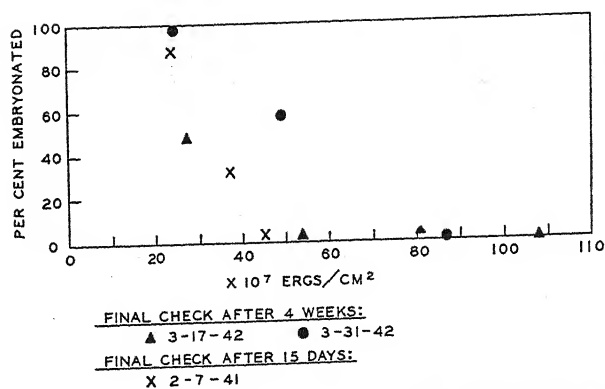


FIG. 4. Effect of 3500 to 4900A radiation on eggs of *Ascaris lumbricoides* in late-segmentation stages.

10 per cent or less of the eggs, whereas within the same time 96 to 99 per cent of the unexposed, control eggs embryonated.

Results of the second series of experiments with one-celled eggs, which were incubated longer, that is for approximately 4 weeks after irradiation, are presented in Fig. 3. Little effect was produced by radiation with energy values up to  $55 \times 10^7$  ergs per cm<sup>2</sup>, and in one test 86 per cent of the eggs embryonated after exposure to  $87 \times 10^7$  ergs per cm<sup>2</sup> although development was delayed; of all the other eggs exposed to radiation of approximately that value or to radiation of greater energy values only 10 per cent or less embryonated. No eggs in 2 of 3 samples exposed to  $123 \times 10^7$  ergs per cm<sup>2</sup> and only a few eggs in a clump near the edge of the dish of the third sample were able to embryonate; unembryonated eggs of these 3 samples remained in the one-cell stage; 98 and 99 per cent of two control samples embryonated.

In the limited tests made, eggs in late-segmentation stages exhibited no greater resistance to irradiation with this wavelength range than did the one-celled forms. Additional testing of this point is indicated since increased development of organisms has been correlated with increased resistance to irradiation with short ultra-violet. Results with three different lots of eggs are presented in Figure 4. Data for embryonated eggs are similar to those for eggs in late segmentation stages. However, as noted above, the criterion of effect of irradiation, that is the activity

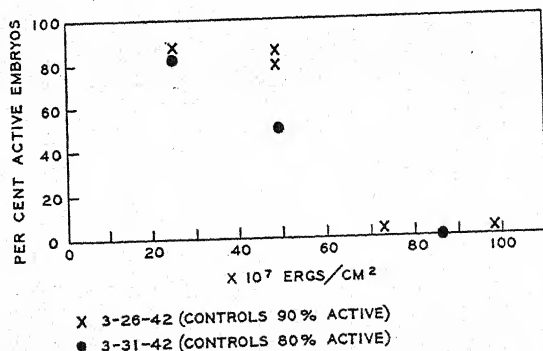


FIG. 5. Effect of 3500 to 4900A radiation on embryonated eggs of *Ascaris lumbricoides*.

of the warmed vermiform embryo is much less satisfactory and the observed results are not exactly comparable with those based on development.

Within individual experiments, in spite of some variability, increased, continued irradiation with sufficient energy effectively reduced the percentage of survival of eggs in any stage of development.

In the few tests in which the resistance of eggs in water and that of dry eggs were compared, there were no marked differences but such differences as there were suggest that the dry eggs may be somewhat less resistant.

A comparison of the rates of embryonation after various exposures is presented in Fig. 6. A delay in development of some irradiated eggs was observed. Such a delay was also apparent in the first few days during early cell division. In one

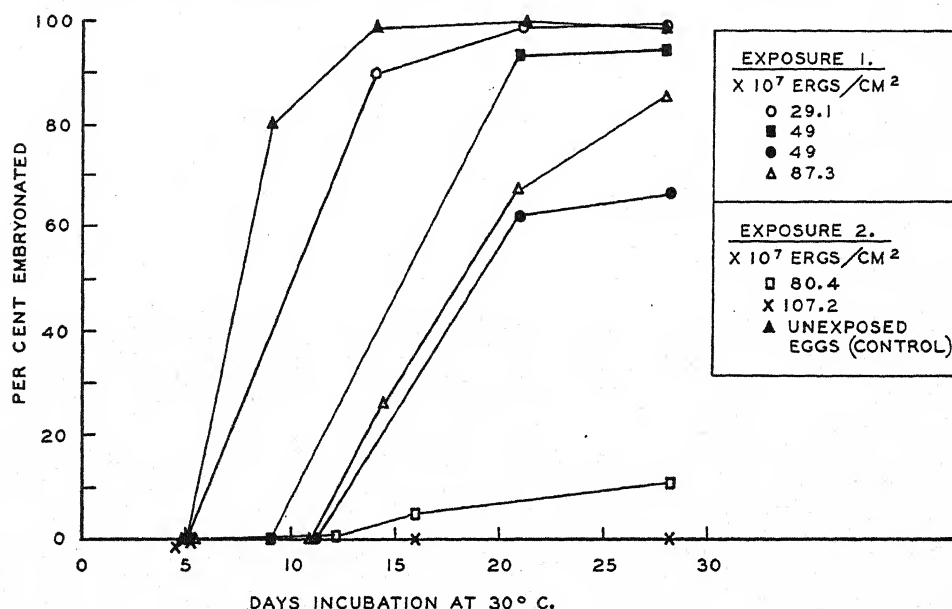


FIG. 6. Rate of embryonation of eggs of *Ascaris lumbricoides* after various exposures to 3500 to 4900A radiation.

experiment, on the fifth day of incubation, control eggs were predominately in 8 and 16-cell stages, eggs exposed to  $29.1 \times 10^7$  ergs per  $\text{cm}^2$  radiation were predominately in 2, 4, and 8-cell stages; eggs exposed to  $87.3 \times 10^7$  ergs per  $\text{cm}^2$  were chiefly undivided, only 1.3 per cent being in 2 and 4-cell stages. In another experiment, on the sixth day of incubation, eggs exposed to  $26.8 \times 10^7$  ergs per  $\text{cm}^2$  were predominately in the 8-cell stage; no eggs which had been exposed to  $80.4 \times 10^7$  ergs per  $\text{cm}^2$  had completed the first cell division, although ultimately 10 per cent became embryonated.

#### DISCUSSION

The energy necessary to produce a damaging effect with radiation in this 3500 to 4900A range is very great, especially if compared to the ultraviolet radiation at wavelengths shorter than 3000A. For eggs of *E. vermicularis*, the energy necessary for lethal action at the wavelengths above 3500A is approximately 1000 times that necessary at wavelengths below 3000A.



In experiments by Wright and McAlister (1934) on eggs of *Toxocara canis* and *Toxascaris leonina*, no apparent effects were noted after exposure to approximately  $27 \times 10^6$  ergs per  $\text{cm}^2$ . These results are comparable to those reported here for eggs of *A. lumbricoides* since consistent, striking effects were observed only after exposures to  $123 \times 10^7$  ergs per  $\text{cm}^2$ , energy which is roughly 50 times that used by the above authors.

The region of the spectrum which was produced experimentally is quite intense in sunlight although the distribution of wavelengths (within the range) is not the same. In sunlight there would be much higher intensity at the longer wavelengths in contrast to this filtered mercury radiation which has the highest intensity around 3650A. Sunlight would contain considerable quantity of infrared radiation and, since higher temperatures appear to enhance the effect of the region used experimentally, sunlight might be expected to be more damaging than the mercury radiation used in this investigation. Sunlight, even though lacking in short ultraviolet, and not necessarily combined with high tropical temperature, should be expected to be lethal for nematode eggs after adequate time of exposure.

The use of sunlight, whenever practicable, would appear to be of value as a factor in the destruction of pinworm eggs and especially so if correlated with high temperature and low humidity.

The results observed in these investigations are to some extent comparable with observations made in Puerto Rico by Spindler (1940) on eggs of the pig ascarid exposed to tropical sunlight at temperatures of approximately 30° to 35° C.

Studies by Hollaender, Cole and Brackett (1939) and Hollaender (1940) on other organisms exposed to the light range used in these experiments have indicated that it might be primarily the respiratory system which is affected. No specific studies with the present material were made as to the method of effect, but nothing in the results of the present study appear to contradict such an interpretation. The delayed development of some irradiated ascarid eggs might be compared to the delayed development which has been observed when they are under conditions of reduced oxygen consumption.

#### SUMMARY

Eggs of the nematodes *Enterobius vermicularis* and *Ascaris lumbricoides* were subjected to long ultraviolet and near visible radiation in the 3500 to 4900A range, exclusive of short ultraviolet and infrared radiation.

Lethal effects were observed after radiation with sufficient energy. The energy necessary in this wavelength range is of different order of magnitude than the energy necessary for comparable effects at wavelengths below 3000A.

A lowered resistance of the eggs of *E. vermicularis* to radiation at higher temperature was observed.

A possible relation of these findings to the observed effects of sunlight is discussed briefly.

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## RESEARCH NOTES

### TRICHOMONAS VAGINALIS IN THE FLUID OF THE VAGINA

Since *T. vaginalis* has usually been studied in a liquid medium (as a rule in some salt solution enriched with serum) we were interested in knowing how it looked and how it behaved in the vagina. With the cooperation of Dr. Weinstein of the Department of Obstetrics and Gynecology of the Medical School of the University of Pennsylvania we procured a small amount of frothy material from the vagina of a negro woman. The specimen was taken with a speculum and a drop of it was examined under the microscope. When it was found to contain many trichomonads it was transferred to a test tube by means of a pipette. The tube was put on a brass inclined plane made for examining tissue cultures in test tubes and with the high dry objective we were able to study the flagellates. The thick creamy white mass consisting of squamous cells, debris and frothy fluid spread out as a long white streak on the wall of the test tube. The trichomonads were swimming freely among the cells and the debris. They had the usual pear shape with a protruding axostyle. The anterior flagella and undulating membrane were active. They varied in size, some being much larger than others. They pushed the cells about just as they do in a salt solution. Clusters of them were seen attached to debris. They were quite plastic and able to push in between the body cells. In fact, in the undiluted material taken from the vagina the trichomonads looked and behaved in all respects like those seen in salt solutions. In other words there was sufficient fluid present in the vagina for them to maintain an active free-swimming existence.—M. J. HOGUE, *University of Pennsylvania Medical School, Philadelphia, Pa.*

### A NEW METHOD FOR QUANTITATIVE ESTIMATION OF MICROFILARIAE IN BLOOD SAMPLES

In counting microfilariae most investigators have employed thick blood films or material recovered from laked and centrifuged blood samples. With the dehemoglobinization technique there is a danger of washing off some of the microfilariae while with the centrifugation method there is a possibility of losing microfilariae in transferring the material. It is believed that the procedure described below obviates such disadvantages.

Twenty cubic millimeters of blood are drawn up into a pipette such as is employed for the hemoglobin estimation by the acid hematin technique. After wiping the tip of the pipette with cotton, the volume of blood is expelled into the chamber of the Sedgwick-Rafter counting cell. This cell was designed for enumerating organisms in water and consists of a slide with a depression 0.1 cm in depth and 2×5 cm in area, thus capable of holding 1 cc of fluid. One cc of 0.1 N hydrochloric acid is added, the suspension stirred with a dissecting needle, and a cover slip applied without leaving an air bubble in the chamber. The microfilariae settle rapidly to the bottom of the chamber and little focusing is thus required. With the aid of a mechanical stage, the entire area of the chamber is examined with the use of a 15 or 25 mm objective.

The method permits the examination of quantities of blood up to 0.1 cc, obviates the possibility of loss of microfilariae in the test sample, and requires only a single piece of equipment. The only disadvantage encountered is that objectives providing magnification higher than 8 mm cannot be used because of the thickness of the preparation.—FREDERICK J. BRADY and ALFRED H. LAWTON, *Division of Zoology, National Institute of Health, U. S. Public Health Service.*

### THE INCIDENCE OF *EMBADOMONAS INTESTINALIS* WENYON AND O'CONNOR IN FOOD HANDLERS AND DIARRHEIC PATIENTS OF MENTAL HOSPITALS

*Embadomonas intestinalis* Wenyon and O'Connor, is considered a rare parasite. Most statistics and surveys do not mention it at all. Contrary to its recorded scarcity, this flagellate was found in 15, or 1.45 per cent of the 1029 examined inmates of State Hospitals of the Greater Chicago area. Of the patients 796 were checked bacteriologically and parasitologically during a survey of food handlers in hospital kitchens and on hospital wards; 233 inmates were examined for diarrheic conditions.

*Embadomonas intestinalis* was found in the stools of 9 or 1.1 per cent of the 796 apparently healthy food handlers. In 6 cases no other parasite was found. The flagellate was accompanied, however, once by *Endolimax nana* and twice by *Endamoeba coli*. Furthermore, the parasite was present in 6 or 2.6 per cent of the 233 diarrheic stool specimens collected from patients showing clinical symptoms of salmonellosis, bacillary or amoebic dysentery. In one case, *Shigella sonnei* was isolated from the feces containing *E. intestinalis*. In a second instance, *Giardia lamblia* and *Endamoeba coli* were found together with *E. intestinalis*. In the third and fourth cases, *Enda-*

*moeba histolytica* accompanied *E. intestinalis*. The stools of the fifth and sixth patients did not reveal other parasites than *E. intestinalis* and no bacterium considered as pathogenic could be cultivated from the same feces.

The frequent findings of *E. intestinalis* in State Hospital inmates can be explained by the greater incidence of intestinal microorganisms of doubtful or proven pathogenicity in chronically institutionalized mental patients, due to the less hygienic habits and housing of such persons on many a State Hospital ward.

The present observations do not warrant ascribing a pathogenic role to *E. intestinalis* but they serve as a stimulus for further investigation of the significance of flagellates in food handlers and diarrheic patients.—VIOLA MAE YOUNG and OSCAR FELSENFELD, *Department of Public Welfare of Illinois and Department of Pathology and Bacteriology of the Chicago Medical School, Chicago 12, Illinois.*

#### OBSERVATIONS ON A SPOROZOAN PARASITE OF THE EELPOUT, *ZOARCES ANGUILLARIS*, WITH AN EVALUATION OF CANDLING METHODS FOR ITS DETECTION

During the recent national campaign encouraging the increased production and utilization of relatively little-used species of marine fishes to help increase the food supply, the eelpout (*Zoarces anguillaris*), heretofore landed only occasionally, was being landed in fair abundance at several New England ports, and prepared for market by filleting. In the course of the increased handling of this fish a protozoan parasite was discovered inhabiting its flesh. A sample of the parasite sent to Dr. Ross F. Nigrelli of the New York Zoological Society was identified by him as belonging to the class Sporozoa, order Haplosporidia, and genus *Ichthyosporidium*.

TABLE 1.—Results of various methods of candling the same 100 eelpout fillets  
(Fish from Cape Cod Bay)

Procedure	Infections in lots of 25 fillets each				Total infected	
	I	II	III	IV	No.	%
<i>Skin on</i>						
No candling—cut side only .....	1	0	1	1	3	3.0
Candling, rapid—cut side only .....	1	1	2	2	6	6.0
Candling, detailed—cut side only .....	1	3	3	5	12	12.0
<i>Skin off</i>						
No candling—both sides .....	1	0	1	1	3	3.0
Candling, rapid—cut side only .....	3	2	2	5	12	12.0
Candling, detailed—cut side only .....	4	3	5	6	18	18.0
Candling, rapid—both sides .....	5	4	6	6	21	21.0
Candling, detailed—both sides .....	11	9	7	7	34	34.0

The parasite, in its trophozoite stage, lies within and parallel to the long axis of the muscle fibers, producing a hyaline degeneration of the latter. The trophozoites, measuring 0.3–5.0 mm in length by 0.3–1.2 mm in width, are readily visible with the unaided eye. They usually lie end-to-end, and in adjacent fibers, one upon another, forming concentrations up to 6–7 cm in diameter. In the great majority of the infected fish the concentrations were close to the vertebral column, in many instances completely surrounding the vertebrae in the region of the infection. The remainder of the concentrations occurred just beneath the skin, mainly near the dorsal and ventral margins of the fish. In most of the parasitized fish the concentrations were anterior to the middle of the body, the largest and oldest being adjacent to the body cavity. All concentrations of trophozoites have a papillae-like appearance. Some of the trophozoites are brownish (usually those in the center of a concentration), while others are whitish (generally at the periphery). No visible morphological difference was noted between these differently pigmented trophozoites. Occasionally, concentrations were observed in which the trophozoites had been partially or completely calcified, forming a hard crust. Apparently, this was due to a host reaction to the presence of the parasite in its tissue. Many fish were observed in which individual trophozoites were scattered throughout or in parts of the flesh, and were not concentrated.

Microscopic examination of several trophozoites revealed that they contained many minute, thick-walled cysts, each of which further contained many egg-shaped spores massed together. The cysts measured 0.011–0.023 mm in length by 0.010–0.019 mm in width, while the individual spores measured 0.004–0.007 mm in length by 0.002–0.004 mm in width. No attempt was made to determine the morphology of the spores.

Examination of 2,032 fillets (representing 1,016 fish) from the area below Cape Cod, extending from Block Island to No Man's Land, revealed that 138 (6.8 per cent) were infected. In the



three samples from this area the per cent of infection varied from 3.9-10.5 per cent. Of the 2,074 fillets (representing 1,037 fish) examined from Cape Cod Bay, 142 (6.8 per cent) were infected. The per cent of infection in the six samples from this area varied from 1.0-34.0 per cent. A small sample of 46 fillets (23 fish) from Casco Bay, Maine contained only one (2.2 per cent) infection. It is significant to note, from the above data covering the examination of 4,152 eelpout fillets during the period from March 24 to August 18, 1943, that some infections were found regardless of the fishing grounds on which the fish were caught.

It was deemed necessary, as an aid in detecting and culling out infected eelpout fillets, to determine the relative effectiveness of various methods of candling these fillets. The candling apparatus employed was the same as that used for rosefish (*Sebastes marinus*) in detecting the presence of, or lesions caused by, the parasitic copepod, *Sphyrion lumpi*. The apparatus consisted essentially of a square or rectangular wooden frame, a lightly frosted plate glass resting on the frame, and two or more electric light bulbs beneath the glass. In candling eelpout fillets commercially, they are pulled rapidly across the surface of the frosted glass, the lights beneath usually exposing in the flesh some or all of the concentrations of trophozoites and scattered individuals. The results of various methods of candling the same lot of eelpout fillets are shown in Table 1. By the rapid candling of both sides of the skinned fillets all concentrations of trophozoites plus some scattered individuals were detected (21 per cent infection). The detailed candling of both sides of the same fillets revealed additional infections (13 per cent more) due solely to the presence of individual trophozoites scattered throughout or in parts of the fillets. All per cents of infection obtained by the methods employed are compared to that of the last method, the latter showing the total per cent of fillets infected.—JACOB H. FISCHTHAL, *U. S. Fish and Wildlife Service, Cambridge, Mass.*

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- No. 1, February 12.
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- No. 3, June 15.
- No. 4, August 10.
- No. 5, October 19.
- No. 6, December 10.

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## FURTHER STUDIES ON THE DEVELOPMENT OF THE SPOROCYST STAGES OF PLAGIORCHIID TREMATODES<sup>1</sup>

W. W. CORT AND D. J. AMEEL<sup>2</sup>

As a part of a program of investigations on the embryology of the digenetic trematodes, a study was made, during the summer of 1941 (Cort and Olivier, 1943b), at the University of Michigan Biological Station of the development of the sporocyst stages of a common plagiorchiid, *Plagiorchis muris* Tanabe, 1922. The material was obtained from immature natural infections in juveniles and adults of *Stagnicola emarginata angulata* (Sowerby) from a beach where a very high incidence of *P. muris* was known to occur.

Only late stages of the mother sporocyst of *P. muris* were found. They are entirely different from any mother sporocysts previously described for this group. They are oval, irregularly disc-shaped masses, 0.5 to 1.7 mm in largest diameter, which are made up of large numbers (about 300 to 500) of closely packed daughter sporocyst embryos (i.e., Figs. 1 and 2). These masses are discrete structures firmly attached to the outside of the snail's intestine, and may occur anywhere along its whole length. Each daughter sporocyst in the mass is surrounded by an outer coat of irregular cells. In fact, the mother sporocyst, at this stage, has no special outer wall, but is composed entirely of the daughters, which appear to be held together by the cells of their outer coats which form the matrix of the mass. This outer coat of the daughter sporocysts persists throughout their whole life. It has been described for mature daughter sporocysts of a number of species of plagiorchiiids, and has been called the paletot.

In the largest mother sporocysts the daughters are elongate and mobile when freed. Each contains cercarial embryos of different stages of development, and a single large discrete germ-mass (i.e., Fig. 13). It was found that when the mother sporocysts are located on those portions of the snail's intestine in front of the digestive gland, the daughters break away from the mother and migrate to the digestive gland and other organs of the snail, leaving behind no trace of the mother. When the daughter sporocysts cease migrating they become firmly attached to the snail's tissue, grow thicker and appear crowded with cercarial embryos. Soon, thickened areas of the true sporocyst wall appear at their ends, which frequently

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<sup>1</sup> A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is one of a series on the embryology of digenetic trematodes in the intermediate hosts. The others have dealt with the strigeids (Cort and Olivier, 1941), the schistosomes (Cort and Olivier, 1943a; Cort, Ameel, and Olivier, 1944) and the plagiorchiiids (Cort and Olivier, 1943b).

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have invaginations at their centers giving the appearance of suckers. From these areas of growth, protrusions push out. Enlargement of these protrusions and their occupation by cercarial embryos produce a variety of irregularly shaped sporocysts, each enclosed in a paletot (l.c., Figs. 18-26). Later, when the period of growth is over, the daughter sporocysts become less irregular in shape because cercarial embryos have come to fill all their length. Growth in size continues after the escape of the first mature cercaria from the terminal birth pore. The germ-mass persists throughout the whole life of the sporocyst, and produces a constant stream of cercarial embryos by a process that has been interpreted as a special type of polyembryony, like that described for the strigeids (Cort and Olivier, 1941). Even in the oldest daughter sporocysts in old snails the germ-masses show no sign of exhaustion and cercarial embryos in all stages of development are present (l.c., Fig. 30).

These observations on the mother sporocyst and the development of the daughter sporocysts of *P. muris* do not fit in with any previous information in the literature on the development of the plagiurchiids. It was difficult to imagine the stages of metamorphosis of the miracidium that would produce the mass of daughter sporocyst embryos which we interpreted as a late stage of the mother sporocyst. No descriptions of sporocysts in this group were found resembling the migrating daughter sporocyst stages; and there were only a few scattered observations that suggested anything like the growth stages. Brooks (1930) stated that germ-masses were present in the daughter sporocysts of the plagiurchiids that he studied, but nothing in his description or figures resembles the germ-masses in these stages of *P. muris*. Certainly, further studies seemed to be badly needed to find earlier stages of the mother sporocysts of *P. muris* and to discover whether the development of other plagiurchiid species in the intermediate host is like this species. Therefore, we decided to continue during the summer of 1942 the study of natural infections of *P. muris* and to extend our observations to other plagiurchiid species.

The present paper, then, covers the observations of the summer of 1942 on the development of the sporocysts of the plagiurchiids. It seems wise to publish them at this time although the information on some of the species is rather fragmentary, since they confirm and supplement the results outlined above already published on *P. muris* (Cort and Olivier, 1943b). Also, it may be some time before it will be possible to undertake further investigations of this type. Altogether, six species of plagiurchiids were studied. By further examinations of *S. e. angulata* we obtained younger stages of the mother sporocysts of *P. muris* and some additional information on its daughter sporocysts. From this same species of snail some data were obtained on sporocyst development in *P. proximus* Barker, 1915, and *P. micracanthos* Macy, 1931. From a limited collection of adults of *Stagnicola palustris elodes* (Say) a few observations were made on the development of another plagiurchiid, *Cercaria talboti* McMullen, 1938. Finally, we examined a considerable series of *Helisoma trivolvis* (Say) and *Helisoma campanulatum smithii* (Baker) and studied the immature infections of *Macroderoides typicus* (Winfield, 1929) and *Alloglossidium corti* (Lamont, 1921) found in these hosts. From these studies, it is clear that in all six species the development of mother and daughter sporocysts has certain fundamental resemblances to that of *P. muris*; also, it is now possible to understand much more clearly the method of development of the mother sporocyst. Starting with the new

information on *P. muris* we will give the results on each species and then discuss the general picture which they give of the mother sporocysts and the development of daughter sporocysts in the superfamily Plagiorchioidea.

*Plagiorchis muris* Tanabe, 1922

An attempt was made in early August of 1942 to find very young mother sporocysts of *P. muris*, by examining a series of juveniles of *S. e. angulata* from the same beach on Burt Lake where the older mother sporocysts of this species had been found during the previous summer. Infections with *P. muris* were evidently entering these snails in large numbers since a large series of mother sporocysts was obtained, including several very early stages.

The youngest mother sporocyst of *P. muris* was a small mass about 0.48 by 0.38 mm in size adhering to the outer surface of the intestine of the snail. Careful manipulation and study under high power of the microscope gave an idea of its composition and made it possible to reconstruct a drawing of an optical section (Fig. 1). It was divided into lobes each containing a number of germ cells. The cells forming the walls were of the same type as those forming the paletots of the individual daughter sporocyst embryos in the older mother sporocysts. Several layers of these same cells formed a base for the attachment of the sporocyst to the outside of the intestine. Some of the compartments containing the germ cells were completely enclosed by the wall while others were only partly enclosed. One other mother sporocyst was found in which only germ cells were present. It was considerably larger, 1.05 by 0.60 mm, and had the same structure as the one figured.

A slightly older mother sporocyst appeared to be divided into several lobes each of which was subdivided into smaller compartments, containing both single germ cells and embryos in the cleavage stages. In some places ingrowths from the wall could be seen which appeared to be the beginnings of still further subdivisions. That is, the whole mother was divided into primary divisions which appeared as lobes, each lobe was subdivided into secondary compartments, and the beginning of tertiary divisions could be seen. In another mother sporocyst in which the subdivision had reached about the same stage, no single germ cells were present but only embryos in the cleavage stages (4- to 16-cell).

In another slightly older mother all the germinal material was in the round "germ ball" stage and the compartments each contained from two or three to several of these embryos. Fig. 2 shows a small part of this mother sporocyst. It shows definite evidence of the growing in of the wall to subdivide the compartments. In some places the wall is more than one layer of cells in thickness. In slightly more advanced stages some compartments contained several free embryos, but most of the daughter sporocyst embryos were surrounded by paletots formed from the wall. In all later stages, the subdividing of the mother by the ingrowing of the wall had been completed and each daughter sporocyst embryo was enclosed in a paletot. Thus the outer layer of cells of the daughter sporocysts, the paletot, which persists throughout their whole lives is clearly seen to be derived from the mother sporocyst wall. Fig. 3 shows a group of daughter sporocyst embryos each surrounded by a paletot. The mother sporocyst from which this drawing was made was about the same stage as the youngest seen during the summer of 1941 (Cort and Olivier, 1943b, Fig. 1). In further development, as described in the earlier paper, the daughter sporocysts



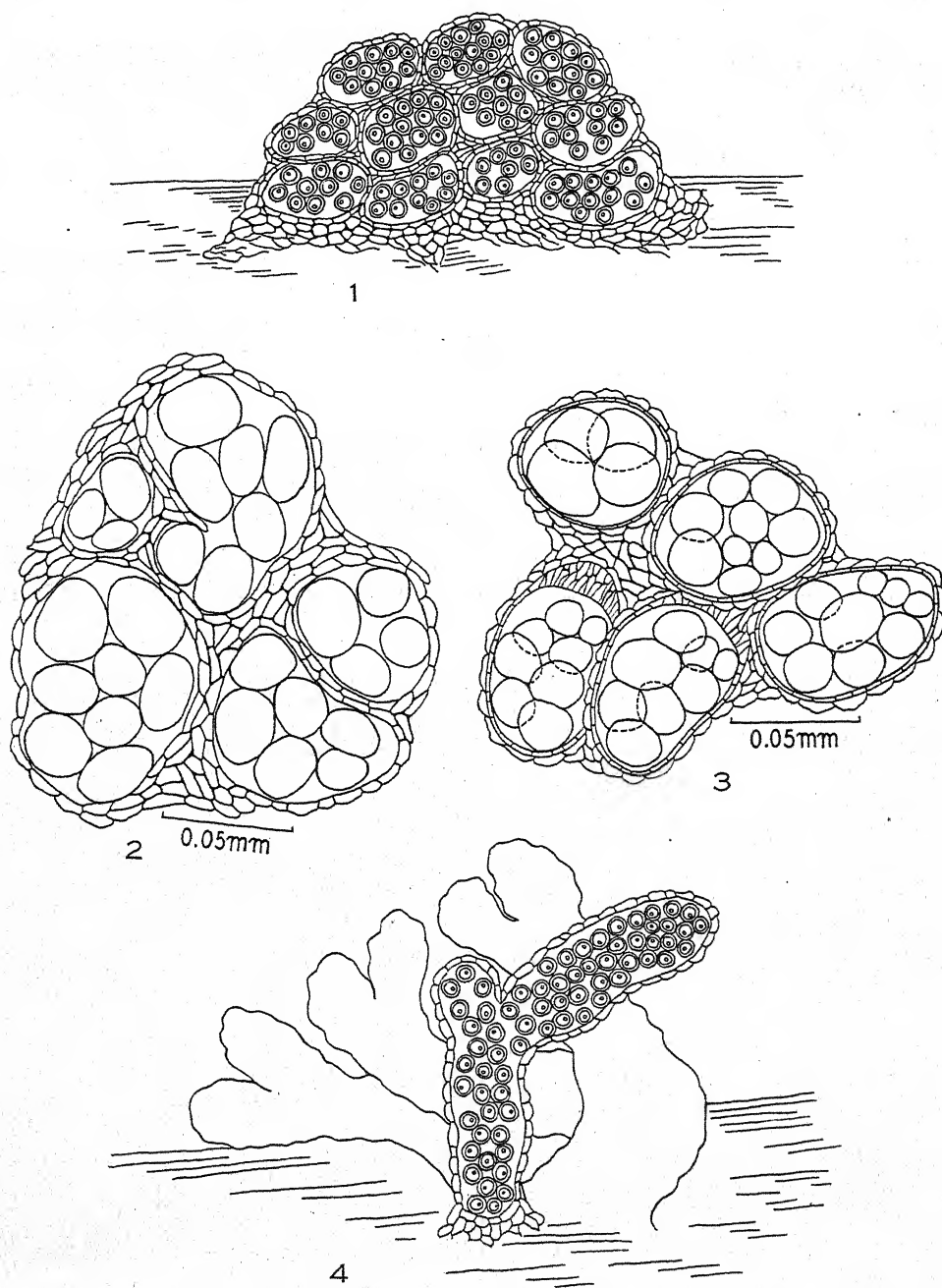


FIG. 1. Very young mother sporocyst of *Plagiorchis muris*.

FIG. 2. A portion of a somewhat older immature mother sporocyst of *Plagiorchis muris*.

FIG. 3. A small portion of an older immature mother sporocyst of *Plagiorchis muris* showing daughter sporocyst embryos already enclosed in their paletots.

FIG. 4. Very immature mother sporocyst of *Plagiorchis proximus*.

elongate and increase in size, until, when they are ready to migrate, their paletots are quite separate, with only a few cells left between them. These observations then confirm those of the previous summer on the mother sporocyst of *P. muris* and show its method of development.

In a number of infections of *P. muris* studied during the summer of 1942, the mother sporocysts were on the parts of the intestine not in contact with the digestive gland. Infections were also found that confirmed the findings of the previous summer on the migrations of the daughter sporocysts from mothers, so located, to the digestive gland. Also, there were numerous cases in which the daughter sporocysts were in the peculiar growth stages. None of the observations in the second summer's work modified the conclusion that asexual reproduction of the daughter sporocysts by fission or budding does not normally occur.

In the summer of 1942, however, we found a number of infections in which the daughter sporocysts were in the growth stages without having migrated from the mother. In such cases it is evident that all chance of migration has been lost. Several of these infections will be described since they show interesting relations between growth and migration in the daughter sporocysts.

In one case a mother sporocyst was attached to the first part of the intestine not far from the stomach. Along its edge daughter sporocysts could be seen that were in process of breaking away; others had already started migration and a few had reached the digestive gland. However, only a part of the daughters was migrating and a large proportion of those still in the mother, perhaps approximately one half, had started to grow without migration. This snail contained no other larval trematode infection.

Another snail had a mature infection of *P. muris* which had the usual distribution all through the digestive gland. In addition there was a mother sporocyst attached to the intestine in front of the gland. The daughter sporocysts of this mother were less numerous than usual and all were in the growth stages. Thus with the liver fully occupied it appeared that the daughter sporocysts of the superimposed infection were developing without migration.

In another snail in which there was a mature infection of *Diplostomum flexicaudum*, a mass was found attached to the intestine in front of the digestive gland. It was composed of mature daughter sporocysts of *P. muris* containing fully developed cercariae. This was interpreted as a case in which the daughter sporocysts had completed their development without migrating from the mother.

In another infection a mass of mature, heavily pigmented daughter sporocysts was found attached to the first part of the intestine just outside the digestive gland. Not a single daughter sporocyst was found in the digestive gland or elsewhere in the tissues of this snail, and there was no other larval trematode infection. This mass had the general shape of a mother sporocyst, but was much larger. It was also interpreted as a case of the completion of the development of the daughters without leaving the mother.

In several infections the mother sporocysts were attached to the part of the intestine in contact with the digestive gland, with all the daughters in growth stages without any having migrated. Also, in one infection the mature daughter sporocysts were limited to a mass on the surface of the digestive gland. In this case the daughters had evidently completed their development in position without any

leaving the mother. In another case such a mass, equal in size to about  $1/5$  that of the digestive gland, was found on the gland with the cercariae almost mature. In some of the cases in which the daughter sporocysts developed without migration there were other larval trematode infections present and in others there were no other infections. Usually when the mother sporocysts were located on the surface of the digestive gland part of the daughters had migrated away from the mother and part had developed without migrations. In such infections the original position of the mother could be determined by finding a solid mass of daughters attached to each other. It is evident, therefore, that migration of the daughter sporocysts of *P. muris* from the mother is not necessary for further growth to take place. Development without migration seems to be rather rare when the mother sporocysts are located in front of the digestive gland, but appears to be the rule for part or even all of the daughter sporocysts when the mothers develop in contact with this gland.

*Plagiorchis micracanthos* Macy, 1931

*P. micracanthos*, the life history of which was worked out by McMullen (1937), is rather rare in *S. e. angulata* in the Douglas Lake region. No immature infections that could be identified as this species were found in our examinations. However, from the few mature infections that were observed, some evidence was obtained that the development of the mother and daughter sporocysts of this species is like that of *P. muris*. In the first place, the daughter sporocysts are very similar to those of *P. muris*, have well developed paletots, and have a similar distribution in the digestive gland and other tissues of the snail host.

One infection of this species was particularly interesting. It consisted of a large, solid mass of mature daughter sporocysts between the foot and digestive gland of a snail. This snail also had a mature infection of *P. muris* with the daughter sporocysts scattered throughout the gland. The mass of mature sporocysts of *P. micracanthos* was a solid discrete structure. It was roughly lens-shaped and the sporocysts which made up its entire structure were so tightly held together that it was removed without breaking. The paletots of these sporocysts were heavily pigmented, giving the whole mass an orange color. These sporocysts contained numerous, active, fully developed cercariae. The interpretation of this mass as a mother sporocyst in which the daughters had developed to maturity without migration seems reasonable, since it resembled very strikingly the cases found in *P. muris* infections in which the daughter sporocysts had completed development without leaving the mother. This certainly suggests that the mother sporocyst of *P. micracanthos* is like that of *P. muris*. Also, the distribution of the daughter sporocysts in other infections of this species suggests that they usually escape from the mother and migrate like those of *P. muris*.

*Cercaria talboti* McMullen, 1938

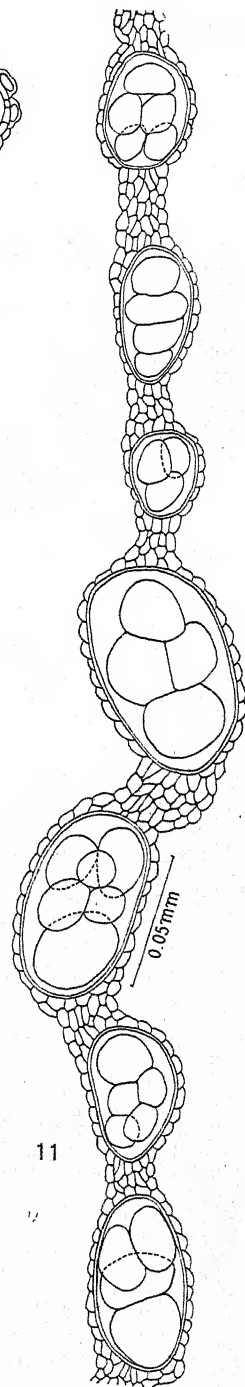
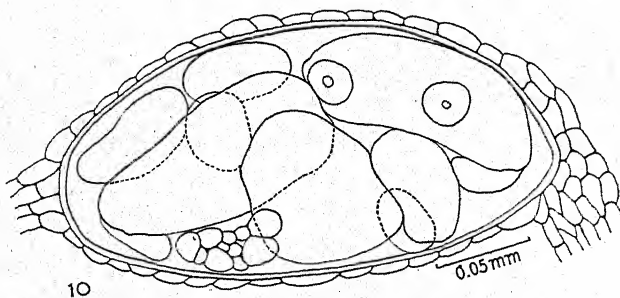
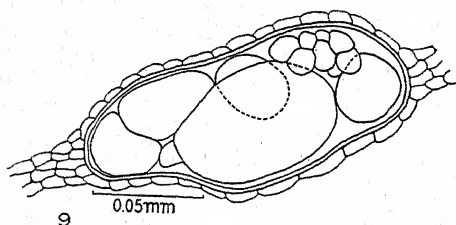
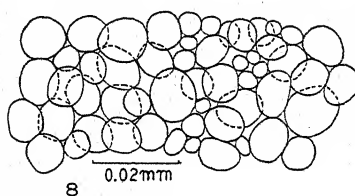
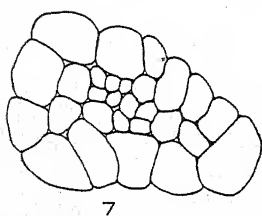
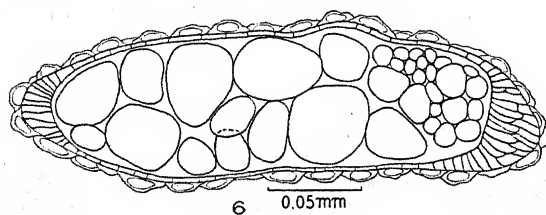
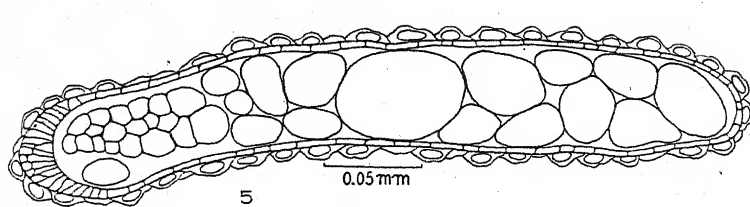
From a collection of *S. p. elodes* from Black Lake, Michigan, we were able to make some observations on the sporocysts of *Cercaria talboti*, which was described by McMullen (1938) from this host and *S. e. angulata*. In several mature and old infections the daughter sporocysts appeared somewhat larger than the measurements given by McMullen (0.795 by 0.188 mm); in one of the oldest infections three of the largest sporocysts had lengths of 1.4, 2.1 and 2.4 mm. The distribution of the

mature daughter sporocysts of this species in the snail host is like that of *P. muris*. They have well-developed paletots containing orange pigment, which is very dense in the oldest infections. Large germ-masses are present, composed of large numbers of both unicellular and multicellular components. These germ-masses are larger than those in the mature daughter sporocysts of *P. muris* and contain more components (Fig. 8). Two that were measured had sizes of 0.17 by 0.07 mm and 0.16 by 0.10 mm. More than one germ-mass was never found in a single daughter sporocyst.

In the snails from this collection two immature plagiorchiid infections were found. They were considered to belong to *C. talboti* since this was the only xiphidiocercaria in this collection and their germ-masses were like those of the mature daughter sporocysts of that species. In one of these infections the daughter sporocysts were in the migrating stage. Part of them was in an irregular mass attached to the snail's intestine not far beyond the stomach. Those at the edge of this mass appeared to be about to migrate. Other daughter sporocysts in the migrating stage were present on and among the organs in front of the digestive gland and all through this gland. Their migration had apparently been recent since none was in the growth stages, and only a few had become attached. The number of daughter sporocysts appeared to be greater than in infections of *P. muris*. A partial count of those still in the mass on the intestine gave 326; and it was estimated that the total number both in the mass and scattered in the tissues may have been a thousand or even more. As seen in Fig. 5, the migrating daughter sporocysts of *C. talboti* are very similar to those of *P. muris*. The cells of the paletot were very distinct, making the surface of the sporocysts quite irregular. Each migrating daughter sporocyst contained from 11 to 18 cercarial embryos and a single germ-mass located at one of the ends. The largest of the cercarial embryos was located at about the middle of the sporocyst, and showed no visible differentiation of organs. These sporocysts were larger than the migrating daughter sporocysts of *P. muris*; eleven that were measured had lengths of from 0.4 to 0.9 mm. Their germ-masses were also distinctly larger than those of the migrating daughter sporocysts of *P. muris*. They had the same structure as in the mature daughter sporocysts but were somewhat smaller; five that were measured had lengths ranging from 0.11 to 0.14 mm, and widths from 0.09 to 0.11 mm. In comparing this immature infection of *C. talboti* with those of *P. muris* at the same stage it seems evident that the method of development must be the same and that the mass from which the daughter sporocysts were migrating must represent the final stages of a mother sporocyst of the same type as that described for *P. muris*.

In the other immature infection of *C. talboti* the daughter sporocysts appeared to be just beyond the migrating stage, and no trace of a mother sporocyst mass remained. They were distributed widely in the digestive gland and also on and among the organs in front of that gland. The cells of the paletot were very prominent and there were distinct thickened areas of the true sporocyst wall at the ends (Fig. 6). These immature daughter sporocysts were attached to the tissues of the host, and were thicker and shorter than the migrating stages of the other infection, the width being about one-third their length. There was also an increase in some of them in the bulk of the germinal material, which appeared more crowded than in the migrating stage (Fig. 5). In such sporocysts the germ-masses were





very prominent, sometimes having a length equal to about one-third that of the body cavity. No growth stages were present in this infection, but the similarity to the pregrowth stages of the daughter sporocysts of *P. muris*, and especially the thickenings of the sporocyst wall at the ends, suggested the same type of growth in the two species. In fact the similarity to *P. muris* was so great that it seems almost certain that the development of the mother and daughter sporocysts of *C. talboti* is like that species.

*Alloglossidium corti* Lamont, 1921

The life cycle of *A. corti* has been studied by McCoy (1928), McMullen (1935) and Crawford (1937). These reports, however, give very little information on the sporocysts. Crawford described the daughter sporocysts as a "mass of much elongated cylindrical sacs twisted around each other." He gave the measurements of a typical sporocyst as 1.8 by 0.16 mm.

In our studies of the development of the sporocysts of this species from *H. trivolvis* and *H. c. smithii* we first examined a number of mature infections. The daughter sporocysts varied considerably in size and shape but were usually more elongate than those of *P. muris*, *P. micracanthos*, and *C. talboti*. In one mature infection seven sporocysts taken at random measured from 0.79 to 2.03 mm in length and from 0.23 to 0.30 mm in width; in another infection twenty were measured, giving lengths of from 0.50 to 2.33 mm and widths from 0.20 to 0.29 mm; the largest sporocyst that was measured from any infection was 3.72 by 0.30 mm. It can be seen from these measurements that the variation in width is small, but that in length is surprisingly great. This shows that average length measurements for the daughter sporocysts of this species would have no significance as a specific character, and suggests that growth occurs chiefly from the ends. As would be expected, the number of active cercariae and developing embryos present in a sporocyst varies with its size; in one small sporocyst, 0.79 by 0.24 mm, 8 active cercariae and four developing embryos were counted, and in one 2.03 by 0.30 mm there were 21 active cercariae.

The germ-masses of the mature daughter sporocysts of *A. corti* are fairly large, complicated structures composed of considerable numbers of both unicellular and multicellular components (Fig. 7). Although they varied considerably in shape, they are roughly triangular, and quite flat. It was difficult to measure or draw them because they were pushed around so much by the active movements of the cercariae. The largest measured had a length of 0.2 mm; another had a size of 0.16 by 0.10 mm and two smaller ones had sizes of 0.13 by 0.05 mm and 0.13 by 0.07

FIG. 5. Daughter sporocyst of *Cercaria talboti* in the migrating stage.

FIG. 6. Immature daughter sporocyst of *Cercaria talboti* just after attachment in the digestive gland of the snail intermediate host.

FIG. 7. The germ mass from a mature daughter sporocyst of *Alloglossidium corti*.

FIG. 8. Camera lucida drawing of an optical section of a germ mass from a mature daughter sporocyst of *Cercaria talboti* to give an idea of the large numbers of components included.

FIG. 9. Immature daughter sporocyst of *Macroderoides typicus* showing the relations of the paletot, the small number of cercarial embryos, and the characteristic germ mass.

FIG. 10. Immature daughter sporocyst of *Macroderoides typicus* at a later stage than Figure 9.

FIG. 11. Showing a portion of a tubule of the mother sporocyst of *Macroderoides typicus* containing immature daughter sporocyst embryos. Over 40 embryos were counted in this one tubule.

mm, respectively. Usually as shown in the drawing (Fig. 7) the multicellular components are around the periphery and the unicellular components are massed in the center. No more than one germ-mass was ever seen in a sporocyst and it was not always easy to find them in the individual sporocysts because of the large numbers of active cercariae and developing embryos that were present.

A well-developed paletot containing orange pigment which is particularly dense in the oldest infections is present around the mature daughter sporocysts of *A. corti*. In most of the sporocysts the end where the birth pore is located is definitely more attenuated than the other. The pore could be clearly seen only when cercariae were escaping. The number of daughter sporocysts in individual infections is very large. In one case 3186 were actually counted, which probably fell far short of the true number.

Although the daughter sporocysts in mature infections of *A. corti* are sometimes found on and among the organs in front of the digestive gland, they appear to be much more localized in this gland than in infections with *P. muris*. Sometimes they are scattered widely through the digestive gland, but frequently they are found in solid masses imbedded in the gland or on its surface. Sometimes, all the sporocysts in the infection would be concentrated in one mass. When the daughter sporocysts are in such masses, they are tightly attached to each other by their paletots and are entirely separate from the tissue of the digestive gland. No masses of mature sporocysts were ever found in front of the digestive glands as was reported above for *P. muris* and *P. micracanthos*. These masses of mature sporocysts of *A. corti* are similar to those sometimes found in or on the digestive gland in *P. muris* infections, which were interpreted as daughters which had developed to maturity without leaving the mother.

The general localization of the mature daughter sporocysts of *A. corti* in the digestive gland of the snail host, and the finding of them in masses in or on the surface of this gland, suggested that the mother sporocysts of this species probably develop on that part of the snail's intestine in contact with the digestive gland. We, therefore, concentrated our search on this section of the intestine and found a considerable series of mother sporocysts. We considered that all of these mother sporocysts belonged to *A. corti*, because in the only other species of plagiiorchiid, *Macroderoides typicus*, that we found in any of the collections of *Helisoma* studied, the mother sporocysts are very different in structure and are always in front of the digestive gland (*vide infra*).

The youngest mother sporocyst of *A. corti*, was a regular rounded mass about 2 mm in diameter, which was attached to the intestine. It was imbedded in the surface of the digestive gland but was pulled away unbroken with the removal of the intestine. When this mother sporocyst was carefully teased apart it was found to contain a large number of very immature daughter sporocyst embryos. These embryos did not appear to be in a series of compartments as in the very young mother sporocysts of *P. muris* but were in a general matrix of mother sporocyst cells. Some of them were in the 6 to 12 cell stages, and the largest were elongate, well-developed sporocysts approaching the migrating stage. The smallest was about 0.027 mm in diameter while the largest was 0.110 by 0.060 mm. All gradations between these two sizes were present. In fact, we found some separate germ cells in a matrix of mother sporocyst cells, but could not be sure that they had not come from small

embryos that had broken up. Very immature embryos in the round "germ ball" stage, only 0.050 to 0.070 mm in diameter, had clearly defined paletots.

Two somewhat larger mother sporocysts of *A. corti* were found in two other snails attached to the intestine after it was pulled away from the digestive gland. They contained daughter sporocyst embryos varying from round "germ balls," 0.06 to 0.07 mm in diameter, to elongate forms up to 0.32 mm in length containing well developed cercarial embryos. All the embryos examined from these two mothers had definite paletots. In the youngest the paletot consisted of only a few of the mother sporocyst cells scattered over the surface, but in some still in the round "germ ball" stage it was a distinct layer. As in the younger mother sporocyst described above, no organization into compartments or tubes could be made out that suggested the method of development.

In a somewhat older infection a part of the mother sporocyst mass was still attached to the intestine after it was pulled away from the digestive gland. Daughter sporocyst embryos in this mass varied from round "germ balls" to elongate forms that were active and ready to migrate. All had definite paletots and germ-masses could be seen in the largest elongate forms, which resembled closely the same stages described for *P. muris*. Some migrating daughter sporocysts were found in the digestive gland at a distance from the mother. Other immature daughters in the gland had become fixed and had started to grow. The growth stages are like those of *P. muris* (Cort and Olivier, 1943b). They include thickened forms with sucker-like growth areas at the ends, forms with small projections from the centers of the growth areas, and others divided into two to three parts showing bizarre shapes. In fact, the shapes depicted for the growth stages of *P. muris* (l.c., Fig. 31) with a variety of modifications could have been drawn from this one infection.

In several other infections of *A. corti* daughter sporocysts in the growth stages were also found. In some cases, it was evident that all the sporocysts had migrated away from the mother before starting to grow. In others, daughters in growth stages were scattered through the digestive gland, and some were in a mass that appeared to be a remnant of the mother. In still others, only the mother mass was present with some or all the daughter sporocysts in the growth stages. For example, in one infection the whole tip of the digestive gland appeared to be made up of a solid mass of immature daughter sporocysts attached to the intestine, most of which were already in the growth stages. Only a very few sporocysts had migrated away from this mass. In another infection the sporocysts were all in one large mass attached to the intestine. Most of them seemed to be in the growth stages, and the cercariae in some of them had their suckers and tails well differentiated.

The evidence from both mature and immature infections of *A. corti* indicates, therefore, that the mother sporocysts of this species develop attached to that part of the snail's intestine that is in contact with the digestive gland. Thus, as they grow in size they tend to become imbedded in the digestive gland. When the daughter sporocysts reach the migrating stage they may leave the mother and establish themselves elsewhere in the gland, and a few may even migrate to tissues in front of the gland. There is, however, a definite tendency for the daughter sporocysts of this species to develop without leaving the mother. This would account for the finding of masses of daughter sporocysts in various stages of growth rather deeply imbedded in the digestive gland but separate from its tissue.



Unfortunately, we did not find early enough stages of the mother sporocysts of *A. corti* to show just how development proceeds. It is evident, however, that this early development must differ from that of *P. muris* since in *A. corti* the daughters in the same mother vary greatly in the stage of development. Also, no evidence was found of the formation of compartments by the growing in of walls. In *A. corti* it appears that the paletots of the daughter sporocysts are formed by the attachment to them of cells of the matrix of the mother sporocyst in which they lie.

*Plagiorchis proximus* Barker, 1915

The larval stages of *P. proximus* are very common and widespread in the Douglas Lake region in *S. e. angulata*. The life cycle of this species was worked out by McMullen (1937) who gave an average size for the daughter sporocysts of 1.021 by 0.243 mm, and noted that they resemble those of *P. muris*. We did not make studies of mature daughter sporocysts of *P. proximus*, except to note that they had well developed pigmented paletots and contained germ-masses. Also, in one case it was noted that sporocysts of this species containing mature cercariae were arranged in strings with the individual sporocysts connected with each other by extensions of the paletots.

In the examinations of *S. e. angulata* during the summers of 1941 and 1942, which were made in connection with the studies on *P. muris*, we found a number of immature plagiurchiid infections in which the daughter sporocysts were arranged in strings which showed branching. Mother sporocysts were also found which were composed of branched tubules containing embryos. All these were considered to belong to *P. proximus* since it was the only plagiurchiid species, besides *P. muris*, that has ever been found in any numbers in this host from the area where the collections had been made. Also, as noted above, mature sporocysts of *P. promixus* had been found in strings. In addition, *P. micracanthus* and *C. talboti*, which have been collected from this host in this area a few times, appear to resemble *P. muris* in the method of mother sporocyst development.

The youngest mother sporocyst which we assigned to *P. proximus* was attached to the snail's intestine in front of the digestive gland. It consisted of four irregular tubules, attached at a common base, each of which had begun to branch dichotomously (Fig. 4). The tubules were about 0.1 mm in diameter, and the whole mass was over 0.5 mm in diameter, which was about the same as the length of each tubule. The walls were composed of a single layer of cells and the same cells formed the base for attachment. These cells are like those that form the wall of the youngest mother sporocyst of *P. muris* described above (Fig. 1). The tubules of this mother sporocyst were packed with large numbers of germ cells which were exactly like those found in the youngest mother sporocysts of *P. muris*. In two places at the ends of the tubules there were small embryos in the early cleavage stages. This was the only very immature mother sporocyst of *P. proximus* that we found. It gives a clue to the method of development of the later stages that were present in considerable numbers.

Several somewhat later stages of the mother sporocysts of *P. proximus* were found attached to the intestine of the snails outside the digestive gland. In them the germinal material was in very early stages of embryonic development and numerous germ cells were still present. These mother sporocysts appeared to be of two types.

A few of them were bushy masses of branching tubules, attached at the base to the intestine. Most, however, were flattened plates extending from the intestine along the mesenteries. These plates appeared to consist of a single layer of branching tubules held together by the cells of their walls, which were later to form the paletots of the daughter sporocysts.

In the youngest of the bush-like mother sporocysts most of the germinal material consisted of germ cells which were packed into the narrow branching tubules in enormous numbers. In a number of the tubules there were early embryos up to about the 16-cell stage. The number of germ cells and embryos appeared to have very greatly increased as compared with the number of germ cells present in the youngest mother sporocyst described above. There seemed to be a great variation in the amount of germinal material present in different immature mother sporocysts, since in one case the germ cells and early embryos were comparatively few and scattered in a linear arrangement along the tubules. Another slightly older bush-like mother sporocyst also had all the germ cells and embryos arranged in linear fashion along the tubules. In it some of the embryos had a distinct sporocyst wall and contained cercarial embryos. When the tubules contained such young daughter sporocysts their walls had become the paletots of the embryos, which appeared as bulges connected by narrower regions. In some of the tubules of this mother sporocyst the germinal material consisted of embryos in cleavage stages arranged in linear fashion and in many of them it consisted only of scattered germ cells.

Most of the mother sporocysts of *P. proximus* which were found attached to the intestines of the snails outside the digestive gland were in plates, one tubule in thickness. In fact, none of the bush-like type contained daughter sporocysts in the later stages of development. It seems possible, therefore, that the mother sporocysts of this species as they develop tend to grow along the mesenteries and assume the flattened plate-like character. In what appeared to be the youngest of the plate-like type there were embryos in the germ ball stage, each surrounded closely by its paletot formed from the tubule wall. At this stage the tubules looked almost like strings of beads. In this mother, numbers of tubules still contained embryos in early cleavage stages, and in some the germinal material consisted only of separate germ cells. Older plate-like mother sporocysts are frequently quite extensive. One showed two distinct parts and extended quite a distance along the intestinal wall and out along the mesentery. Along its edges were daughter sporocysts, with a well developed wall, which contained cercarial embryos. Elsewhere the tubules were much narrower and contained only cleavage stages or separate germ cells.

Other older plate-like mother sporocysts had daughters, especially along the edges, in the migrating stage. One such mother, on the last part of the intestine outside the digestive gland, was irregularly oval in outline and migrating daughter sporocysts could be seen breaking away from its margin. It also contained younger embryos in all stages of development and in some of the smaller tubules there were only germ cells. Two mother sporocysts, attached to the intestine outside the digestive gland, even contained daughters in growth stages like those described for *P. muris* (Cort and Olivier, 1943b).

A few mother sporocysts of *P. proximus* were found attached to the portion of the intestine imbedded in the digestive gland. These appeared as masses of branching tubules with embryos in various stages of development. In one case such a mass

at the base of the digestive gland contained large numbers of daughter sporocysts already in growth stages. Although more observations are needed, it seems clear that in *P. proximus* daughter sporocysts may either migrate from the mother, or the growth stages may take place without migration. Very striking also in the mother sporocysts of *P. proximus* is the great variation in the stages of development of the embryos, since migrating daughters, and in a few cases even growth stages, and all earlier stages of embryonic development are found in sporocysts in which some tubules still contain germ cells.

*Macroderoides typicus* Winfield, 1929

In his discussion on the life cycle of *M. typicus*, McMullen (1935) gave no information on the sporocyst stages except that the mature daughter sporocysts have a size of about 0.36 to 0.13 mm and contain 1 to 4 cercariae. Our studies on the development of the sporocysts of this species were made of natural infections in *H. trivolvis* and *H. c. smithii*.

In mature infections the daughter sporocysts are in large orange-colored masses lying among the organs between the foot and digestive gland of the snails. In occasional infections the sporocyst mass would be in contact with the base of this gland, would somewhat overlap its proximal part or would even give the appearance of being partly imbedded in it. Never, however, are the sporocysts actually in the tissues of the digestive gland.

The daughter sporocysts of this species are comparatively small and in the mature infections the orange pigment in the paletot is unusually dense. When the masses of mature sporocysts were detached from the snail's organs and teased apart with needles they were usually found to be in strings, attached end to end by extensions of their paletots. Sometimes in the densest masses the arrangement into strings is not evident. The sporocysts themselves are by far the smallest that were studied and are irregularly oval with a slightly pointed end at which the birth pore is located. Measurements of twenty from one infection gave lengths ranging from 0.48 to 0.87 mm and widths from 0.20 to 0.36 mm. In other infections some were considerably smaller and some were larger. The two largest sporocysts that were seen in any of the numerous infections observed measured 1.34 by 0.23 mm and 0.95 by 0.23 mm, respectively. The sizes of the masses and the number of daughter sporocysts in them vary greatly in different infections. In one of the larger infections in which an attempt was made to count the sporocysts the number was found to be approximately 4000.

The number of mature and developing cercariae in the daughter sporocysts of *M. typicus* is much less than for any other of the plagiurchiid species in our series, varying from 5 to 12 in eleven sporocysts from one infection; of these from 1 to 4 were mature or almost mature cercariae. In addition, each daughter sporocyst contains a small, somewhat flattened germ-mass of rather regular outline. It was not always easy to find these germ-masses because the contents of the sporocysts were moved about by the activity of the cercariae and they had to be carefully observed to distinguish them from cercarial embryos. The germ-masses in this species are the smallest of any of the plagiurchiid species studied and the number of their components is much less. Seven that were measured within the living sporocysts had a range in length of from 0.070 to 0.105 mm and a width from 0.049 to 0.059 mm.

Even in the oldest infections, as already noted, some sporocysts are attached end to end in strings by extensions of their paletots. Daughter sporocyst embryos in the round "germ ball" stage are occasionally present in infections containing mature cercariae. Also, the same string may contain a few small, immature daughter sporocysts and others very much larger with mature or almost mature cercariae. Occasionally very long strings of a large number of sporocysts could be isolated. Even the very longest strings were unbranched. Figs. 9 and 10 show two stages of immature daughter sporocysts from such strings.

The finding of these strings containing immature daughter sporocysts led us to look carefully for young infections in which none of the cercariae was mature. Three immature infections were found at about the same stage of development in which the youngest embryos were small round "germ balls" and the largest although well formed sporocysts contained no cercariae with differentiated body and tail. These infections, which consisted of a mass of tubules or strings attached to the enlargement of the esophagus of the snails next to the stomach, were interpreted as mother sporocysts. From the central mass of the mother sporocyst strings extended out to considerable lengths reaching the nearby organs of the snail. The examination of these strings showed that they consisted of tubules of paletot cells enclosing at intervals the developing daughter sporocyst embryos. Some of them were surprisingly long; in one 40 daughter sporocyst embryos at various stages of development were counted (Fig. 11). We had the impression that some of the strings were even much longer than this. In no instance was any branching of these tubules seen.

A still younger mother sporocyst was found in another snail, also located on the bulb formed at the point where the esophagus enters the stomach. It was composed of three small masses of the tubules or strings radiating from a common center. All that could be made out when this whole mass was detached and observed under the microscope were large numbers of unbranched tubules of paletot cells each containing a linear series of the daughter sporocyst embryos. The oldest daughter sporocyst embryos in this mother had well-formed walls and the youngest were in early cleavage stages. Although careful search was made no germ cells were seen. At the tips of some of the strings, embryos in the cleavage stages or in the early "germ ball" stage were present, and the paletot cells could be seen to form definite tubules along which the embryos were scattered. In those parts of the strings where the embryos had grown to immature sporocysts, in which the wall was well formed and the cercarial embryos were distinct, the walls of the tubule adhered closely to the sporocysts forming their paletots.

#### DISCUSSION

The observations recorded in this and the previous paper (Cort and Olivier, 1943b) on the development of the plagiurchiids gives an entirely new conception of the mother and daughter sporocyst development in this group. In all of the six species studied the daughter sporocysts retain throughout their whole lives an outer layer of cells, the paletot, which is derived from the mother sporocyst. Characteristic also is the formation of large numbers of daughter sporocysts by multiplication of the cells of the germinal line in the early stages of the development of the mother sporocysts. Then, in the early development of the secondary germinal sacs, the



daughter sporocysts, there is first a limited multiplication of germ cells followed by the formation of a single germ-mass for each daughter. These germ-masses produce a constant stream of cercarial embryos throughout the whole life of the daughter sporocysts by the breaking off of multicellular components in a special type of polyembryony. The combination of the extensive multiplication of the cells of the germinal line in the mother sporocysts which is interpreted as a primary polyembryony of the zygote (fertilized ovum) with the secondary polyembryony of the germ-masses of the daughters, produces the enormous numbers of cercariae so characteristic of the plagiorchids. In a species like *A. corti* where the number of daughter sporocysts is unusually great, and each daughter is large, it seems possible that more than a million cercariae can be produced from one fertilized ovum.

Another striking point in the development of these plagiorchids is the almost unlimited growth of the cells forming the wall of the mother sporocyst which later form the paletots of all the daughter sporocysts. These cells appear to come from the "subepithelial" layer of cells of the miracidium which forms the wall of the young mother sporocyst. Although the wall of the mother sporocyst increases greatly in extent in other groups to form the comparatively huge sacs of the strigeids or schistosomes, its almost unlimited growth with the formation of the paletots of the daughter sporocysts is characteristic of the plagiorchids.

Another characteristic of this group is the peculiar method of growth employed by the daughter sporocysts after they have reached their final position in the snail's tissue. While this was seen in only three of the six species studied it probably occurs in all but *M. typicus*. This growth, consisting of the extension of the walls of the sporocysts from growth areas at their ends, produces stages with very bizarre shapes, which sometimes suggest multiplication by budding or fission. However, there is no evidence from our observations that this kind of asexual multiplication of daughter sporocysts normally occurs.

The discussion given above indicates the general pattern of plagiorchid development as shown by the six species studied. The most striking differences among them are in the structure of the mother sporocysts and in the methods by which the daughters are distributed to the tissues of the snail. In these respects the six species appear to show four different types.

The mother sporocyst of *P. muris* illustrates the first type, to which those of *P. micracanthos* and *C. talboti* also appear to belong. The youngest mother sporocyst of *P. muris* observed (Fig. 1) already was divided into compartments by the ingrowth of the cells of the wall. Each compartment contained a number of separate germ cells. By later ingrowths of the wall the mother sporocyst of this type is subdivided into smaller and smaller compartments, until finally each embryo is surrounded by a part of the wall, the paletot. The oldest mother sporocysts consist, therefore, of a mass composed of large numbers of elongate motile daughter sporocysts each surrounded by its part of the mother sporocyst wall. Then, when these daughters migrate, the mother completely disappears since practically all of the cells of its wall have been used in the formation of the paletots of the daughter sporocysts. One of the most striking characteristics of the mother sporocyst of the *P. muris* type is that all the daughters are at about the same stage of development. This synchronous development apparently is brought about by all the germ cells starting to develop into embryos at about the same time after the completion of a definite phase

of germ cell multiplication. Also, it seems characteristic of this type for the daughters to migrate from the mother, especially to all parts of the digestive gland of the snail. However, occasionally in mother sporocysts outside the digestive gland and more frequently in those imbedded in the gland some or all of the daughters undergo the characteristic growth stages and develop to maturity without migration, producing masses of mature daughters. It is possible to suggest that in *P. muris* the miracidium penetrates the intestine and metamorphoses on its outside into a simple sac containing a few germ cells enclosed in the layer of cells derived from the body wall of the miracidium. Then as this sac grows and the germ cells multiply, division comes about by ingrowths of the body wall, until finally each of the daughters which develop from the germ cells is surrounded by a portion of the mother sporocyst wall which forms its paletot.

A second type of mother sporocyst development was found in *A. corti*. In this case the developing daughter sporocyst embryos appear to lie in a matrix of mother sporocyst cells, from which each receives its paletot when in the round "germ ball" stage. No definite division of the mother sporocyst into compartments is present, and the embryos in a single mother vary considerably in the degree of development. The mother sporocysts in this species are always imbedded in the digestive gland of the snail, and while there was evidence of migration it appears that the daughters very frequently undergo the growth stages, which are like those of *P. muris*, without leaving the mother. We have no evidence on the method of metamorphosis of this type of mother sporocyst from the miracidium, since early stages were not found, but it is evident that the phase of multiplication of the germ cells must continue for a considerably longer time than in *P. muris* to produce the variation in the development of the embryos found in one mother sporocyst.

The development of the mother sporocyst of *P. proximus*, which is considered as the third type, appears at first glance to be entirely different from *P. muris*, since the daughters come to lie in branching tubules which form plate-like layers, and the paletots of the embryo daughter sporocysts are formed in an entirely different fashion from either *P. muris* or *A. corti*. The youngest mother sporocyst of *P. proximus* that we found (Fig. 4) gives a clue to the method of formation of the mother sporocyst of this type. It might be suggested that a simple sac containing a few germ cells is first formed by the metamorphosis of the miracidium. Simple outgrowths from this sac would early form the stage shown in Fig. 4. Continued branching of these outgrowths and elongation of the branches would produce the later stages. Characteristic of this type of mother sporocyst is the formation of plates composed of a single layer of branched tubules. At first the germ cells are massed in the tubules. Later these cells and the embryos they form become strung along the tubules. The period of germ cell multiplication must last even longer than in the development of the mother sporocyst of *A. corti* since in a single mother sporocyst all stages of development from germ cells to migrating daughter sporocysts may be present. Entirely different, also, is the method of formation of the paletots of the daughter sporocysts. As the embryos grow, that part of the tubule in which they lie comes to adhere closely to their outer wall so that when they have become elongate daughter sporocysts the tubule wall has become their outer layer or paletot. Thus are formed strings of daughter sporocysts connected by those portions of the tubules between them. When these daughter sporocysts undergo the

growth stages without migration their attachment to each other by the extensions of their paletots may still persist after they have become mature. It seems probable that when the mother sporocysts of this type develop in front of the digestive gland, the daughters usually break away from the mother and migrate to the digestive gland. They do, however, sometimes develop in mothers outside the gland without migration. As in *P. muris*, it would be expected that in mothers that develop in the tissues of the digestive gland, growth without migration would be more frequent. However, our observations on this point are very limited.

The development of the sporocysts of *M. typicus* is considered as a fourth type. In this species the daughter sporocysts are always found in front of the digestive gland. It appears that they do not migrate from the mother sporocyst and do not go through the peculiar growth stages that are characteristic of the other three types. The very immature mother sporocysts of this species are small masses of unbranched tubules attached to a common base on the outside of the bulb of the esophagus of the snails next to the stomach. Within the tubules there is a linear series of daughter sporocyst embryos, which vary from early cleavage stages to daughter sporocyst embryos with a well-developed wall and separate cercarial embryos. As indicated by Fig. 11 the paletots of the daughter sporocysts are formed by the walls of the tubules in the same way as in the mother sporocysts of *P. proximus*. Also, the variations in the developmental stages of the daughter sporocyst embryos found in a single mother indicates that in *M. typicus* there is a considerable lag in the development of the germ cells.

Although no very immature mother sporocysts were found for *M. typicus* it is possible to suggest the course of development. After the metamorphosis of the miracidium into a simple sac containing a few germ cells, there must be very extensive tubular outgrowths from the mother sporocyst wall. There is no evidence that these tubules ever form secondary branches. An extensive multiplication of germ cells must occur and these germ cells are carried along with the tubular outgrowths, and come to lie at intervals along them. As individual germ cells develop into embryos the walls of the tubule adhere to them and form the paletots. The extensive growth of the tubules and the development to maturity of the daughter sporocysts would finally form the masses of daughter sporocysts of the mature infections. A mature infection of *M. typicus*, therefore, is merely a very greatly enlarged mother sporocyst from which the daughters have never escaped. This failure to migrate from the mother means that the daughters are localized in front of the digestive gland and are never found in the tissues of this gland. It appears also that the growth of the daughter sporocysts of this species does not include the peculiar stages where protrusions are produced from areas of growth at the ends as in the other three types. The daughter sporocysts of *M. typicus* are, therefore, the smallest of any studied and contain the smallest number of cercarial embryos.

In spite of the considerable variations shown in these six species in the structure of the mother sporocyst and the development of the daughter sporocysts they all show fundamental relationships in their germ cell cycle and general development. The six species belong to two quite different families of the Plagiorchioidea, i.e., the Plagiorchiidae and the Macroderoididae. That this same general type of development occurs in a wide variety of other plagiorchiids is suggested also by the description of paletots for the daughter sporocysts of a number of different species (cf. Cort



and Olivier, 1943b). It is rather surprising, therefore, that the mother sporocysts of plagiorchids described in the literature are quite different in structure from any of the four types we have found. Three of these are of species of the Reniferidae (Talbot, 1933; Byrd, 1935; Walker, 1939) and the other is of *Plagitura parva* described by Stunkard (1936). In all these species the mother sporocysts are described as thin-walled sacs which are similar to this same stage of other digenetic trematodes. The outer coat or paletot is not shown for the daughter sporocysts of these species. It would appear, therefore, that there are wide variations in the structure of the mother sporocysts in the Plagiorchioidea, and that further knowledge of this stage may be of value in developing a more natural classification.

#### SUMMARY

Observations on the mother sporocysts and development of the daughter sporocysts in immature natural infections of six different plagiorchid species, *Plagiorchis muris* Tanabe, 1922, *P. micracanthos* Macy, 1931, *Cercaria talboti* McMullen, 1938, *Alloglossidium corti* (Lamont, 1921), *P. proximus* Barker, 1915, and *Macroderoides typicus* (Winfield, 1929), has given an entirely new conception of the structure and development of the sporocyst stages in this group. In all these forms the daughter sporocysts have an outer covering of cells, which forms a distinct outer layer, the paletot, which is derived from the wall of the mother sporocyst, and which persists throughout the whole life of the daughters. Characteristic also is a phase of multiplication of the cells of the germinal line, which may be interpreted as a polyembryony of the zygote, in the early development of the mother sporocyst which produces large numbers of daughter sporocysts. In the daughter sporocyst embryos after a limited period of multiplication of the cells of the germinal line, germ-masses are produced, only one for each daughter. These produce cercarial embryos throughout the whole life of the daughter sporocysts by the breaking off of multicellular components in a secondary polyembryony. This combination of extensive multiplication of cells of the germinal line, which may produce from several hundred to several thousand daughter sporocysts, with the extraordinary reproductive activity of the germ-masses in the daughter sporocysts, produces the enormous numbers of cercariae so characteristic of the plagiorchids.

Another striking point is the almost unlimited growth of the cells of the mother sporocyst wall to form the paletots of the daughter sporocyst. These paletots in some species are formed by the ingrowing of the wall of the mother sporocyst and in others from the walls of tubular outgrowths of the mother sporocysts in which the developing daughters are enclosed.

Another characteristic of the plagiorchid species studied is the growth of the daughter sporocysts after they had reached their final position in the snail host by protrusions of the wall from growth areas at the ends. This results in stages showing very bizarre shapes, which in some cases suggest multiplication by budding or fission. However, there is no evidence from our observations that this type of asexual multiplication normally occurs.

Finally, the distribution of the daughter sporocysts to the various tissues of the snail, especially the digestive gland, usually occurs by the migration of daughter sporocysts from the mother. However, not infrequently the daughters go through the growth stages and develop to maturity without leaving the mother sporocyst.



This most frequently occurs when the mother sporocysts develop in the digestive gland. In one of the species, *Macroderoides typicus*, the daughters never leave the mother and appear to develop to maturity without going through the characteristic growth stages. Whether the daughters leave the mother sporocyst or not they retain throughout their whole life as an outer layer, the paletot, a portion of the wall of the mother.

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## METHODS FOR COLLECTING TICKS FOR STUDY AND DELINEATION<sup>1</sup>

EDWARD T. BOARDMAN<sup>2</sup>

Several years ago the author became dissatisfied with the traditional solution of 70 per cent alcohol commonly used for killing as well as preserving ticks and other arthropods. A tick killed in strong alcohol dies with its legs so contracted as to obscure important diagnostic characters. For this reason, a solution was found which killed ticks with their legs outstretched.

In addition to developing a new killing solution, several modifications of collecting techniques were evolved to meet conditions in the southern and southeastern states.

The author is indebted to the late Dr. George H. F. Nuttall, of the University of Cambridge, for suggesting the use of a tightly closed bag for carrying small vertebrate hosts parasitized by ticks. The bag as originally proposed has been modified as indicated below. To Dr. H. E. Ewing, of the U. S. National Museum, credit is due for suggesting the use of glycerine-alcohol in homeopathic, cork-stoppered vials for permanent preservation of arthropods.

### METHODS OF COLLECTION

Ticks can be collected from vegetation (Kohls, 1937) by using a white cotton flannel flag which is about one yard square. The author has modified this flag by adding a strip of material so as to make two long shallow pockets down the middle of each face. A four-inch strip of cotton flannel is fastened onto the flag by means of a seam down its center. The ends of the strips are then sewn down to complete the pockets. Ticks tend to lodge in these and consequently do not fall off.

A flag seems to be relatively ineffective in the southern states where the ground is warm and ticks are less inclined to climb up into the sunshine. There ticks can sometimes be collected most effectively from the ground litter along animal trails, around dens and in bedding-down areas. At other times, the best results are obtained from walking cross-country with a large dog. The dog should be encouraged to range widely and be allowed to choose his own resting spots. Ticks will be found concentrated around the neck and ears of the dog if they are allowed time to attach. The collector's person and clothes will also be a source of ticks.

When native hosts are trapped or shot, they should be examined minutely as soon as possible after death. In order to do this, it is best to lay the carcass on a white cloth or paper so that any ticks that leave the host will be seen.

For convenience in the field, small host animals can be placed in bags as soon as killed. Preferably, the warm body should be put in a cotton flannel bag which is closed by means of a drawstring. The seam for the drawstring should contain extra material to form a ruffle within the mouth of the bag. This ruffle serves to prevent

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the escape of ticks which crawl from the chilling host. In addition, a wad of absorbent cotton should be inserted as a plug in the mouth of the bag as the draw-string is pulled tight. Most of the ticks will fall off into this bag.

Attached ticks should have their mouthparts dissected free of the host skin if wanted alive, otherwise, they should be wetted with kerosene or some other thin oil (Imes, 1926). Oiled ticks will drop off within a half hour and will die.

#### METHOD OF KILLING

The composition of the collecting fluid is important. Although it has been customary to collect many terrestrial arthropods into 70 per cent alcohol, the author has long preferred a solution of ninety-seven parts of 20 per cent alcohol and three parts of ether. Ticks usually spread their legs to the utmost before dying in this solution. This permits clear inspection of coxal spines and other ventral characters used in tick identification. These are usually covered by the curled-up legs of ticks which have been killed in strong alcohol.

Ticks which have been killed in this ether-alcohol solution are better for teaching purposes because their appearance is more nearly life-like and because their external anatomy is readily observed.

This technique has proven successful with ticks, hydroacarina, immature and adult aquatic insects, and many terrestrial arthropods. The container should be large enough to allow large species to die outstretched.

#### FINAL PRESERVATION

Since ether-alcohol is not a satisfactory preservative, the fluid should be changed as soon as possible. The author uses stoppers tinted with colored ink for his collecting vials. As soon as the fluid in the vials is replaced with the preserving solution, stoppers of the usual color are substituted.

Final preservation is in a solution containing nine parts of 75 per cent alcohol to one part of glycerine. The glycerine is insurance against desiccation in case the alcohol evaporates. Specimens are permanently stored in homeopathic vials with oversize cork stoppers rolled down to fit.

Although this method is not as desirable, all but engorged ticks can be removed from the killing solution, dried rapidly, and stored in pill boxes or other appropriate containers, between sheets of cellucotton or soft paper which has been suitably padded.

#### RENDERING CHITINIZED STRUCTURES VISIBLE

The presence of glycerine has one disadvantage. Clear, chitinized structures, such as the coxal spines and the teeth of the hypostome and of the chelicerae of ticks become almost invisible. For detailed examination of these parts the ticks must be washed repeatedly in fresh 70 per cent alcohol and viewed by lateral illumination. If the parts are still hard to see, they can be stained with dilute aniline dyes, such as methylene blue. A more satisfactory staining is obtained by using Galigher's modification of Bethe's method for testing for chitin (Galigher, 1934), which is as follows:

#### MODIFIED BETHE'S METHOD FOR STAINING CHITIN

1. Transfer the tick from clean, glycerine-free alcohol to a solution containing 95 cc. of 90 per cent alcohol and 5 cc. of concentrated nitric acid. Material should remain in this mixture—3–5 days.

2. Wash in three changes of 50 per cent alcohol.
3. Place ticks for three minutes in freshly prepared 10 per cent aqueous solution of aniline hydrochloride, to which one drop of hydrochloric acid has been added to each 10 cc. of solution.
4. Rinse in water.
5. Place in 10 per cent aqueous solution of potassium bichromate until stained an intense grass green.
6. Rinse in water.
7. Place in water containing one drop of ammonium hydroxide per every 10 cc. The color will soon change to blue.
8. Wash in water for five minutes and return the specimen to 70 per cent alcohol.

Note:—Steps 3 and 5 may be shortened or protracted according to the size of the structures to be stained. The parts should be stained a clear, semi-transparent blue.

#### SUMMARY

1. Methods are described for collecting ticks from soil, vegetation and host animals.
2. It is important for diagnostic purposes that ticks be killed in an expanded condition. The author has found that a solution of 3 parts of ether and 97 parts of 20 per cent ethyl alcohol will kill ticks with their appendages expanded.
3. This ether-alcohol solution has proven useful in killing many different arthropods, both aquatic and terrestrial.
4. Preferably ticks and other arthropods should be transferred to a solution of one part glycerine and nine parts of 75 per cent alcohol for final storage, however, the more highly chitinized species may be dried.
5. An outline of a modified Bethe's method for staining chitin is suggested for studies of small, clear, chitinized structures.

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*EIMERIA BREVOORTIANA*, A NEW SPOROZOAN PARASITE  
FROM MENHADEN (*BREVOORTIA TYRANNUS*), WITH  
OBSERVATIONS ON ITS LIFE HISTORY

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INTRODUCTION

Most of the species of the genus *Eimeria* are intestinal parasites throughout their life histories. In a few instances oöcysts have been found in various other organs of the host animal, such as kidney, spleen, liver, air bladder and testis. With the exception of one, *Eimeria stiedae* (Lindemann), which is parasitic in the liver of rabbits, the life histories of these species are incompletely known. Any information, therefore, which helps explain their peculiar behavior will no doubt be of value in determining the relationships of other members of the genus and of the genus itself.

During the course of an investigation of the parasites of marine fishes, oöcysts of an eimerian parasite were found in the testes of menhaden. Two parasites of this nature have previously been described from the testes of fishes, *E. sardinae* (Thélohan, 1890) from sardines, and *E. nishin* Fujita (1934) from herring. In these cases, as with the present species, the only stages seen in the testes were those that belonged to the sporogonic cycle, zygotes, sporocysts, and sporozoites.

Since the typical life history of an eimerian includes a schizogonic as well as a sporogonic cycle, a study was begun to determine the location of the former in menhaden. Smears and sections of testes, stomach, pyloric ceca, intestine, spleen, liver and kidney were examined. Eventually, structures resembling the schizogonic stages of other eimerians were found in the epithelium of the pyloric ceca—spores, schizonts, macro- and microgametocytes. Together with the cycle of sporogony in the testes they form a complete life history of an eimerian, which is described in the following pages.

The writer is indebted to many friends for help during the course of the work, particularly to Dr. A. S. Pearse of Duke University, under whose direction it was carried out. Dr. H. F. Prytherch kindly extended the facilities of the United States Fish and Wildlife Service Laboratory at Beaufort, North Carolina. Capt. Chas. Hatsell and many fishermen of Beaufort helped in collecting material, and Margaret Hardcastle aided the writer greatly in the examination of much of it. Dr. D. C. Boughton of the United States Bureau of Animal Industry read the manuscript and made many helpful suggestions.

MATERIALS AND METHODS

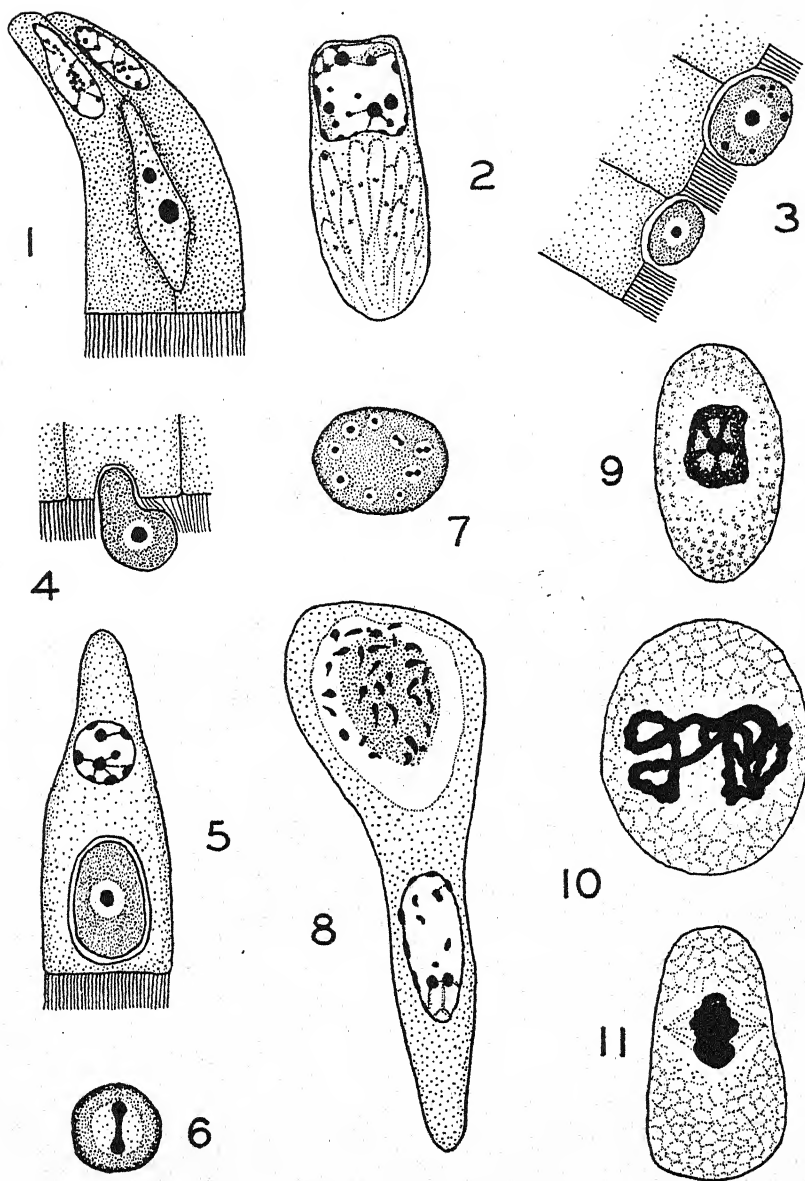
All material was collected at Beaufort, North Carolina, during the years 1937–1942. Collections included specimens from various parts of the coast, at all seasons of the year.

During the early part of the work it was found essential that material for the

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- FIG. 1. Developing trophozoite in epithelium of ceca.  
 FIG. 2. Mature schizont.  
 FIG. 3. Young gametocyte in ciliated border of cecal epithelium.  
 FIG. 4. Young gametocyte penetrating epithelial cell.  
 FIG. 5. Intracellular gametocyte.  
 FIGS. 6-7. Division of nucleus of microgametocyte.  
 FIG. 8. Nearly mature microgametocyte in host cell.  
 FIG. 9. Macrogametocyte.  
 FIGS. 10-11. Division of macrogamete or zygote.

study of intracellular stages be absolutely fresh. Since this necessitated fixation of tissue in the field, Bouin's fluid was used almost exclusively, with satisfactory results. Tissues were embedded and sectioned in the usual manner. Schizogonic stages in the ceca were stained with Heidenhain's iron hematoxylin and counter-stained with light green. Ehrlich's hematoxylin gave best results with testicular tissue.

Fresh oöcysts were obtained by the same methods generally used to collect them from feces. Portions of testis were thoroughly pulverized in a little water, strained through cheesecloth, centrifuged, and the oöcysts floated with a concentrated salt solution. All measurements were made in a hanging drop suspended over a depression slide.

For the study of sporocysts and sporozoites, oöcysts were crushed between a coverslip and slide. The resulting smear was fixed in Schaudinn's fluid and stained with Ehrlich's hematoxylin.

All drawings were made with the aid of a camera lucida. Oöcysts were drawn from fresh material to an original magnification of 660 $\times$ ; all other drawings were made from stained sections or smears to an original magnification of 1350 $\times$ .

#### LIFE HISTORY

##### *Trophozoites*

The earliest stages of the parasite seen in the ceca were trophozoites that had penetrated the epithelium lining (Fig. 1). These were rarely seen, and only in isolated groups. They are cigar-shaped structures measuring about 12 microns in length by about 2.5 microns in width. In the center is a large nucleus, which in the early stages is a round solid mass of chromatin. At the anterior end is a mass similar to, but smaller than, the nucleus, presumably a refractive globule. In most of the trophozoites a bulge is present near the center, so that hardly any two of them are of the same size and shape. It seems likely that they develop rapidly after entering cells.

##### *Schizonts*

As the schizont enlarges the nucleus divides into small fragments which separate. Hazy lines appear in the cytoplasm and each encloses a fragment of the nucleus to form a merozoite.

Mature merozoites measure 3 to 5 microns in length by 0.5 to 1 micron in width. Usually 8 to 10 are present in a single schizont. Their anterior end is slightly enlarged and the other tapers to a slender tail which extends toward the outer end of the cell. In the center a nucleus is barely visible.

Mature schizonts (Fig. 2) average 12 microns in length by 6 microns in width, although their size is extremely variable. They may be compressed into long slender structures, or be shortened and almost round, depending probably on the pressure of surrounding cells.

Nothing definite can be said about the number of generations of schizonts. The merozoites could not be measured accurately enough to detect differences in size, and smears from fresh material were not successful. However, the fact that schizonts are by far the most commonly seen stage would seem to indicate that more than one generation is produced.

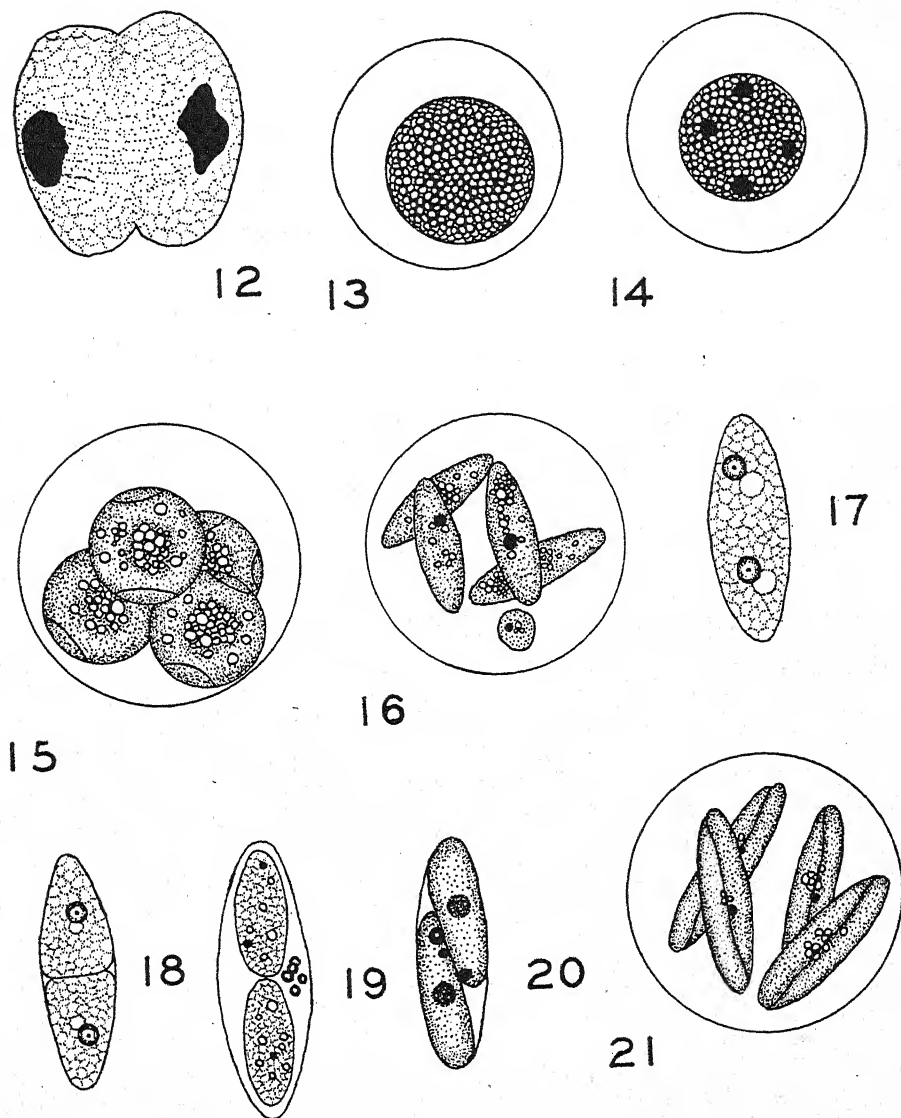


FIG. 12. Later stage in division of macrogamete or zygote.  
 FIG. 13. Oöcyst containing undifferentiated zygote.  
 FIG. 14. Division of zygote nucleus.  
 FIG. 15. Oöcyst containing sporoblasts.  
 FIG. 16. Oöcyst containing spores.  
 FIGS. 17-20. Formation of sporozoites.  
 FIG. 21. Fully sporulated oöcyst.



*Microgametocytes*

Since the merozoites are of such small size, the earliest gametocytes are barely visible under a magnification of 1250. They are almost invariably found lodged in the ciliated border of the epithelial cells (Fig. 3). Possibly some of the merozoites themselves penetrate cells, but gametocytes usually found there (Fig. 5) are in a rather advanced state of development. Their appearance is typical. Each consists of a granular cytoplasm that takes a fairly heavy stain, and surrounds a perfectly clear area. In the center of this area is a small, round, heavily staining mass of chromatin. The young gametocytes of both sexes are identical, as far as could be determined. The cytoplasm is essentially similar and granules may be found scattered through each.

The gametocytes generally develop in the ciliated border of the host's intestinal epithelium until a diameter of about 5 microns is reached. From this size they may continue their development extracellularly, but the majority bore into adjacent cells, apparently by ameboid movement (Fig. 4). The significance of this behavior will be discussed later.

After entering a cell, development follows the usual procedure. The nucleus divides and subdivides until the whole structure is filled with daughter nuclei. Each then begins to elongate, finally reaching a length of about 2 microns (Figs. 6 and 7). Flagella have not been seen but it is assumed that they are present.

When maturity is reached, the microgametocyte is about 12 microns in diameter and fills the outer half of the host cell (Fig. 8). The microgametes are scattered about over the exterior, and as they leave, the greater part of the microgametocyte remains as a residual mass in the cell.

*Macrogametocytes*

Macrogametocytes can be identified by the time they have reached a diameter of 5 microns. They greatly outnumber the microgametocytes, in fact, with the exception of schizonts, they are the most common stage encountered.

Macrogametocytes also can probably complete their development in the ciliated border of the cecal epithelium, but seldom do so. When they enter a cell they barely get inside and take up a position between the nucleus and the outer border; only in rare instances have they, or any stage, been seen in the basal portion. In the cell they continue to enlarge until a diameter of about 10 microns is reached. At this time the macrogametocyte undergoes a reorganization. The cytoplasm becomes more homogeneous, the clear area about the nucleus disappears (Fig. 9), and eventually a complete division takes place (Figs. 10-12), resulting in two daughter cells.

These events are not at all clear, due in part to the impossibility of getting a complete series of stages, and in part to the fact that no information could be obtained as to when fertilization occurs. It seems probable that the macrogamete is fertilized just before, or during, its reorganization. If such is true, the division may be a reduction division which involves the whole zygote, resulting in two migratory post-zygotic stages, or oökinetes, which will carry the infection to the testes.

*Oöcysts*

All of the stages described thus far occur in the host's pyloric ceca, while those that follow are in the testes. It will be assumed for the present that the second cycle follows immediately after the first, and discussion of this point will be reserved until later.

The earliest stage seen in the testes is a typical oöcyst, which consists of a fully formed cyst wall that encloses a granular protoplasmic mass (Fig. 13). Some of the oöcysts are very much smaller (12 microns) than a fully sporulated oöcyst (25 microns), and the enclosed mass completely fills the interior. About 700 testes, in all stages of development have been searched for an earlier stage than this, but nothing has been seen which in any way might be so interpreted.

In some species of *Eimeria* it is believed that the protoplasm shrinks away from the cyst wall during development, creating a space between. In this species the size of the protoplasmic mass apparently remains about the same; those measured varied from 15 to 19 microns in diameter, with an average diameter of 15.7 microns in both large and small oöcysts. The average diameter of the oöcysts that contain fully developed zygotes is 22.59 microns, compared to an average diameter of 25.09 microns for fully sporulated oöcysts. This appears to indicate that enlargement of the cyst wall, rather than shrinkage of the protoplasm, takes place.

In the fresh material the central mass is composed of small indistinct granules of stored food, enclosed by a membrane. No nucleus is visible. In stained sections the granules are quite distinct, and present a rather loose arrangement. The nucleus appears as a dark central area, and usually is in some stage of division. This apparently is a lengthy process, as zygotes just on the verge of division have been kept under observation for 12 hours without any sign of change.

The first division results in two nuclei which take up positions on opposite sides of the zygote. These in turn divide and produce four nuclei which migrate to the periphery to form the corners of a square (Fig. 14). The cytoplasm then divides to produce four sporoblasts and a residual body.

The sporoblasts (Fig. 15) are circular and measure about 10 microns in diameter. Each has its own share of food material and a portion of chromatin. The latter, in most cases, does not seem to reorganize but immediately begins the nuclear division that will form the sporozoites.

Fully mature spores (Fig. 16) average 16.4 microns in length by 6.3 microns in width. In fresh material they appear to be filled with granules of various sizes, similar to the undifferentiated zygote. In stained sections they show a slightly granular cytoplasm with two nuclei, one at each end, and a vacuole close by each (Fig. 17). A karyosome is present in each nucleus.

The division of the spore into sporozoites is quite interesting. The spore elongates somewhat and the protoplasm begins to pinch in two transversely at the center (Fig. 18). This occurs inside the sporocyst, which does not itself divide. The protoplasm finally becomes completely divided and each half begins to pull apart and to separate from the sporocyst, producing two oval bodies which rest end to end (Fig. 19). A small sporocystic residuum is also formed during the division. The oval sporozoites now begin to elongate and grow more slender, eventually filling the sporocyst (Fig. 20). They are cigar-shaped, slightly curved structures with one end pointed and the other blunt. A large nucleus is present in the center. When mature (Fig. 21) they average 15.0 microns in length by 2.5 microns in width. In the sporocyst they lie side by side, slightly curved about each other, and face in opposite directions. With completion of this process the oöcyst is fully formed and ready to infect a new host.

The oöcysts, like those of many other species, occur in both spherical and oval

shapes. The round type is by far the most common, and ranges in diameter from 17.50 to 30.00 microns, with a mean diameter of 25.09 microns. Range in size of the oval oöcysts is  $21.20-30.00 \times 15.00-27.50$  microns, with a mean size of  $26.16 \times 22.73$ .

Of the 700 fishes examined, 41.9 per cent of the males were infected. This figure is lowered considerably by the infrequency of infection in younger fishes; infection often runs as high as 100 per cent in a large group of mature fishes. No parasites were ever seen in the ovaries of females, although the schizogonous stages occur in the ceca. They either do not continue their development or it occurs in some other part of the body.

#### DIAGNOSIS

*Eimeria brevoortiana* n. sp., parasitic in the testes of menhaden, *Brevoortia tyrannus* (Latrobe). Oöcysts occur in round and oval forms. From 300 measurements the range in diameter of round oöcysts was 17.50–30.00 microns, with a mean diameter of 25.09 microns; range in size of oval oöcysts,  $21.20-30.00 \times 15.00-27.50$  microns, with a mean size of  $26.16 \times 22.73$ . Oöcystic and sporocystic residua present. No micropyle present. *E. brevoortiana* is differentiated from *E. sardinae* and *E. nishin*, also parasitic in the testes of fishes, by the much greater size of the latter (45–50 microns).

#### DISCUSSION

The life history of *Eimeria brevoortiana*, here set forth, exhibits one fundamental difference from that described for other members of this genus—schizogony and sporogony occur in different organs of the host. This difference, at first consideration, appears to be a radical departure from the usual procedure in which sporogony follows schizogony in the gut without interruption. Such a life history as the one described, however, might well be expected when certain species with similar characteristics are considered. *E. truncata* (Railliet and Lucet) parasitizes the kidneys of geese, *E. gadi* Fiebiger the swimbladder of cod fish, *E. kermoganti* (Simond) the spleen of gavials, and *E. sardinae* (Thélohan) and *E. nishin* Fujita the testes of fishes. The way in which these errant coccidians gain access to the organs they parasitize is not yet known, so it seems quite certain that some members assigned to this genus have life histories that do not conform to the types now known.

In the present case, the fact that stages typical of an eimerian parasite have been found in the testes, suggests that schizogony occurs at least in the same host, if not in the same organ. Seven hundred testes have been examined, at all seasons of the year and in all stages of development, and there is no evidence whatever to indicate that any stages of schizogony occur in them. As mentioned previously, other organs likely to harbor such stages have been examined with negative results. Since the stages in the gut are typical and are the ones required, they are believed to be parts of the life history of the same parasite.

On the other hand, the schizogonic phase found in the pyloric ceca would normally be followed by a sporogonic phase, in which oöcysts would be produced and liberated in the feces. To test this possibility, the intestinal contents of 170 fishes were examined at all seasons of the year but no oöcysts were found. This is strong evidence that such are not present.

Ordinarily a question such as this could be answered conclusively by feeding infective stages to parasite-free hosts and tracing their development. In the case of

menhaden, however, their fragile nature, and the impossibility of securing parasite-free specimens precluded such experiments.

As to the means by which the parasite reaches the testes, there are three possibilities—the blood stream, through the cloaca, or penetration of the intervening tissues. Regarding the first, Smetana (1933) has demonstrated that the sporozoites of *E. stiedae* (Lindemann) are hatched in the gut of rabbits and that they bore through the intestinal walls and reach the liver by way of the portal veins. The fact that one member of the genus *Eimeria* employs the blood stream as a means of transportation suggests that *E. brevoortiana* may use the same method.

Halawani (1930) found oöcysts of *E. southwelli* in the spiral valve of intrauterine devil-fish embryos, and suggested that the parasite had traveled down to the cloaca of the mother and ascended the uterus. The vasa deferentia of many menhaden were examined with this in mind, but no zygotes or earlier stages were ever found there.

It seems more likely that the testes may be reached by active migration through the tissues. As previously mentioned, the early gametocytes begin their development in the ciliated border of the epithelium and later appear to bore into a cell, as illustrated in Fig. 4. This behavior is quite interesting. In some species such as *E. gadi* Fiebiger (1913) and *E. mitraria* (Laveran and Mesnil, 1902), the entire development of the parasite takes place extracellularly. In contrast to these, the great majority of eimerians are intracellular parasites. There is another group, however, to which *E. brevoortiana* belongs, which exhibits a transition from one type to the other. *E. anguillae* Léger and Holland (1922) is extracellular in development, but when the macrogametocyte matures it bores in to the base of the epithelium and there sporulates. *E. pigra* Léger and Bory (1932) is extracellular as a rule, but occasionally certain of the stages enter cells. Léger and Bory regard this as the beginning of an adaptation to intracellular life.

The fact that the early gametocytes of *E. brevoortiana* are motile, suggests the possibility that they may be motile at other stages as well. This seems plausible in light of the observations of Elmassian (1909) on the macrogametocytes of *E. rouxi*. He writes—"le *Coccidium* (= *Eimeria*) *rouxi* est essentiellement migrateur et cela toutes les périodes et toutes les phases de sa vie, même quand il revêt la forme de cellule sexuelle. . . . Cela surtout est vrai pour les gamètes qui avant d'atteindre le terme de leur développement ont besoin de se rechercher en vue de la fécondation." He states that he has actually seen the macrogametocytes boring through the epithelium of the host. In consideration of these facts, and observations on the present species, it is believed that in the case of *E. brevoortiana*, infection is carried from the gut to the testes by a motile macrogamete or zygote by active penetration of the tissues.

The question also arises as to how and when infection takes place. Spawning of menhaden, from the meager accounts available, takes place off shore, where schools of fish congregate and shed their eggs and milt into the water. In a few mature males which have been examined, sporulated oöcysts were found in the vasa deferentia and mixed with the sperm stripped from the testes. If they are passed into the water in this way, they could easily be picked up by other hosts, since menhaden are plankton feeders and have numerous long gill rakers which serve as a very efficient straining apparatus.



## SUMMARY

A new species of sporozoan parasite, *Eimeria brevoortiana*, has been described from the menhaden, *Brevoortiana tyrannus* (Latrobe). All stages of sporogony were seen in the testes and these are described. No evidence of schizogony was seen in 700 testes examined.

All stages of schizogony characteristic of members of the genus *Eimeria* were found in the epithelium of the pyloric ceca of menhaden and these are described. No such stages could be found in other organs of the host. The intestinal contents of 170 menhaden were examined but no oöcysts were found.

From the evidence at hand it appears logical to assume (1) that the two cycles are continuous, and are parts of the life history of the same parasite; (2) that infection of the gut is carried to the testes of the same host by a migratory stage; (3) that infection of other hosts takes place by oral ingestion of oöcysts which have passed into the water with sperm from infected hosts.

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# ACQUIRED RESISTANCE IN CHICKENS, TURKEYS, AND RING-NECKED PHEASANTS TO THE GAPEWORM, *SYNGAMUS TRACHEA*

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A number of investigators have reported observations on the resistance of chickens to the poultry gapeworm, *Syngamus trachea*. Ransom (1921) showed experimentally that as chickens grow older they become increasingly resistant to gapeworm infection. Clapham (1934) reported that 13-week-old chickens are refractory to infection, while Waite (1920) was successful in infecting two of three yearling hens by feeding them large numbers of earthworms infected with the larval forms of these gapeworms. Ransom (1921) infected 5 of 17 mature chickens to which large numbers of embryonated eggs and larvae of this nematode had been previously given. Morgan (1931) found one yearling hen infected with 12 pairs of worms, and Crawford (1940) observed that it was not unusual to find adult chickens infected with gapeworms in Ceylon. The latter investigator stated that "the number of pairs of worms found in the tracheas of adult fowls is generally small, as a rule not more than two or three pairs, but not infrequently adult hens have been seen on poultry farms exhibiting typical clinical symptoms. . . ." Olivier (1943) observed that 7 of a flock of 13 mature chickens were infected with gapeworms following exposure to contaminated soil at the Beltsville Research Center, Beltsville, Md. One of these birds showed typical symptoms and harbored 94 pairs of worms at necropsy.

Clapham (1934) concluded that diets low in vitamin A or in minerals, particularly calcium, reduced the resistance of 13-week-old chickens to *Syngamus*.

Whitlock (1937) observed that female partridges were more susceptible to *Syngamus* infection than the male birds. Clapham (1939) also observed this sex difference in susceptibility of partridges and noted that the young birds had heavier infections than the older birds, although the latter were not immune.

No account of investigations on acquired resistance to *Syngamus* in turkeys and pheasants appears to have been reported other than an abstract (Olivier, 1942) of some of the material contained in this paper. It is generally considered that an intimate association between host and parasite is necessary for the induction of an immune response in the host. The larvae of *S. trachea* penetrate the tissues of the host and also live for a time in the lungs. As adults they are attached to the tracheal mucosa. Therefore, it was suspected that an acquired resistance to this parasite might be induced in susceptible birds, and the six experiments described in this paper were undertaken to investigate that possibility.

## MATERIALS AND METHODS

All the birds were obtained as one-day-old chicks, and were held under parasite-free conditions until used in the experiments. The Rhode Island Red chickens and the White Holland, Bronze, and Beltsville Small White turkeys were supplied by the Animal Husbandry Division, Beltsville Research Center, Beltsville, Md. The

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pheasants were obtained from the Loyalsock State Game Farm, Loyalsock, Pennsylvania, through the kindness of Mr. Richard Gerstell, Chief, Division of Propagation and Research, Pennsylvania State Game Commission.

Material for inoculation of birds was prepared by teasing apart in water the uteri of mature gapeworms taken from turkeys. The cultures were held at room temperature until the eggs had embryonated. Soon after becoming embryonated, many of the eggs hatched. Under refrigeration the freed larvae remained active and infective for several weeks. Consequently, the inoculating material consisted of both embryonated eggs and hatched larvae, usually in the ratio of about two of the former to one of the latter.

The infective material for each of the experiments was obtained by mixing several individual cultures, none of which was over three months old. The concentration of eggs and larvae was determined by counts of a 0.15-cc culture sample. The measured dose of eggs and larvae was introduced into the esophagus of the bird near the crop by means of a long, calibrated pipette.

Four to six weeks following the first administration of infective material, a second dose of eggs and larvae was given to determine whether the birds had acquired resistance. The birds were autopsied two weeks after the second inoculation. This time interval was selected in order (1) to allow sufficient time for the worms of the second inoculation, if any, to develop and reach the trachea and to establish themselves there, and (2) to make it easier to differentiate, on the basis of size and degree of development, between the worms of the two inoculations. Observations showed the worms of the second inoculation were much smaller and the males less firmly attached to the tracheal wall; their heads were not embedded in a nodule as in the case of the males developing from the first infection.

#### EXPERIMENTAL DATA

##### *Turkeys*

*Experiment I (see Table 1).*—Each of 20 turkeys, seven weeks old, was given 10,000 embryonated eggs and larvae of *Syngamus* on June 24. Five of these birds died between the 19th and 25th days after inoculation, and, at necropsy, they harbored an average of 235 pairs of worms per bird. Groups I and III in the table represent the 15 surviving birds. Thirty-seven days<sup>1</sup> after the infective material was administered the birds of group III were autopsied to determine the approximate number of worms per bird prior to administration of the second dose. An average of 22.6 pairs of worms per bird was found in the seven birds. On August 4 the birds of group I (previously infected) and eight uninfected birds of the same age (group II) were each fed 5,000 eggs and larvae to determine their comparative resistance. At autopsy 15 days later, group I birds had an average of 2.3 pairs of worms of the first infection but harbored no worms of the test infection, while group II birds harbored an average of 107.9 pairs of worms per bird.

*Experiment II (see Table 1).*—Three groups (I, II and III) of turkeys, 40 days old, were fed 2,500 embryonated eggs and larvae per bird on September 17; group IV was held as controls. The birds of group I were autopsied two weeks after inoculation, and the birds of group II were autopsied four weeks after inoculation. An average of 24.1 pairs of worms per bird was recovered from group I and an average of

<sup>1</sup> In a previous report (Olivier, 1942) this figure was erroneously given as 41 days.

19.3 pairs of worms per bird was recovered from group II. On October 15, the birds of group III (previously inoculated) and group IV (uninfected) were each given 5,000 eggs and larvae. Two weeks later the birds of both of these groups were autopsied; those of group III harbored an average of 15 pairs of worms per bird of the original infection and an average of 2.1 pairs per bird of the test infection. How-

TABLE 1.—Results of experiments on resistance of young turkeys, ring-necked pheasants, and chickens to infection with *Syngamus trachea*

Group No.	No. of birds	First inoculation of eggs and larvae (number)	Av. No. worms per bird two weeks after inoculation (pairs)	Av. No. worms per bird four weeks after inoculation (pairs)	Total No. worms at autopsy	Test inoculation of eggs and larvae (number)	Pairs of worms per bird at autopsy	
							Av. No. first infection	Av. No. second infection
Experiment I (Young turkeys)								
I	8	10,000	...	...	.....	5,000	2.3	0
II	8	.....	...	...	.....	5,000	...	107.9
III	7	10,000	...	22.6	.....	....	...	....
Experiment II (Young turkeys)								
I	16	2,500	24.1	...	.....	....	...	....
II	16	2,500	...	19.3	.....	....	...	....
III	16	2,500	...	...	.....	5,000	15.0	2.1
IV	15	.....	...	...	.....	5,000	...	54.4
Experiment III (Ring-necked pheasants)								
I	17	3,000	...	...	.....	5,300	0.0	0.7
II	5	.....	...	...	.....	5,300	...	94.0
III	5	3,000	...	...	One dead pair. Two living males	....	...	....
Experiment IV (Chickens)								
I	12	2,500	7.6	...	.....	....	...	....
II	12	2,500	...	0.7	.....	....	...	....
III	11	2,500	...	...	.....	5,000	0.2	0.0
IV	12	.....	...	...	.....	5,000	...	0.7
Experiment V (Chickens)								
I	24	2,100	10.1	...	.....	....	...	....
II	24	2,100	...	0.9	.....	....	...	....
III	24	2,100	...	...	.....	3,000	0.45	0.0
IV	24	.....	...	...	.....	3,000	...	5.4
Experiment VI (Chickens)								
I	24	1,500	10.8	...	.....	....	...	....
II	24	1,500	...	5.6	.....	....	...	....
III	24	1,500	...	...	.....	4,100	0.6	3.5*
IV	24	.....	...	...	.....	4,100	...	3.3

\* Twelve of the 13 birds harbored no worms of the second infection. The remaining bird harbored 45 pairs.

ever, no worms of the second infection were recovered from 11 of the 16 birds of this group. On the other hand, the birds of group IV harbored an average of 54.4 pairs of worms per bird of the second infection, all the birds being infected. The difference in the number of worms of the second infection recovered from the two groups is highly significant, since the difference between the means is 5.4 times its standard error.



The results of these two experiments show clearly that turkeys developed a marked resistance to infection with *S. trachea* as a result of a single previous infection. In the first experiment the previously infected birds were negative at necropsy for worms of the test infection while the uninfected controls were heavily infected. In the second experiment the previously infected birds acquired only a few worms of the test infection while the controls became heavily infected. In the birds of both experiments worms of the immunizing infection were present when the test dose was administered.

#### *Ring-necked Pheasants*

*Experiment III (see Table 1).*—Each of fifty pheasants, 39 days old, was fed 3,000 embryonated eggs and hatched larvae on July 14. Twenty-eight of these birds died between the 13th and 27th days after inoculation, and an average of 74 pairs of gapeworms per bird was recovered at necropsy. The 17 birds of group I and the five birds of group III listed in table I represent the survivors. The birds of group II were treated like those of the other two groups except that they were not given the immunizing dose. Forty-one days after inoculation the birds of group III were autopsied and only one dead pair and two living males from which the females had become detached and lost were recovered. The following day each of the birds of group I and II was fed 5,300 eggs and larvae. At autopsy 13 days later, 12 worms, all of the second infection, were recovered from 7 of the 17 birds of group I; 10 of the birds of this group harbored no worms. The controls, however, were all infected, harboring 470 pairs of worms of the second infection, or an average of 94 pairs per bird. This difference is 6.3 times its standard error.

The results of this experiment demonstrate that pheasants developed a marked resistance as a result of heavy infections with *S. trachea*. At autopsy, the previously infected birds had no worms, or only very few, following the second dose. On the other hand, the uninfected birds became heavily infected following the second dose. Since the birds of group III had lost almost all of their worms as revealed by a post-mortem examination on August 24, it is probable that the birds of group I were almost worm-free when given the second dose on August 25. Nevertheless, they showed a high degree of resistance to reinfection. It appears, therefore, that the resistance developed by these birds persisted after the worms which induced the resistance were lost.

#### *Chickens*

*Experiment IV (see Table 1).*—Two groups (I and II) of 12 birds each and one group (III) of 11 chickens, 45 days old, were each given 2,500 embryonated eggs and hatched larvae on September 17. Another lot of 12 birds (group IV) was held as controls. At necropsy two weeks after inoculation the birds of group I harbored an average of 7.6 pairs of worms each; four birds had no worms at all. Four weeks after inoculation the birds of group II harbored an average of 0.7 worm per bird; eight of these birds had no worms. On October 15, each of the birds of groups III and IV was fed 5,000 eggs and larvae. Two weeks later the birds of group III harbored a total of two pairs of worms of the first infection and no worms of the second infection. The controls harbored eight pairs of the second infection, or an average of 0.7 pair per bird. Eight of the birds in this group had no worms.

*Experiment V (see Table 1).*—Ninety-six chickens, 21 days old, were divided

into four groups. The birds of groups I, II and III were each fed 2,100 embryonated eggs and hatched larvae on December 27. Those of group IV were held as controls. The birds of group I, when autopsied two weeks later, harbored an average of 10.1 pairs of worms per bird; six of the birds had no worms. An average of 0.9 pair of worms per bird was found in the birds of group II when autopsied four weeks after inoculation; eight of these birds had no worms. On January 27, the remaining birds of group III (11 died following the first inoculation) and each of group IV was fed 3,000 eggs and larvae. Twelve days later both groups were autopsied. The birds of group III harbored an average of 0.5 pair of worms of the original infection and none of the second infection. The controls harbored an average of 5.4 pairs of worms per bird; three of them had no worms.

*Experiment VI (see Table 1).*—Ninety-six chickens, 21 days old, were divided into four groups. The birds of group I, II and III were each fed 1,500 embryonated eggs and hatched larvae on January 27. Those of group IV served as controls. The birds of group I were autopsied on February 11 and were found to harbor an average of 10.8 pairs of worms per bird; one of the birds had no worms. An average of 5.6 pairs of worms per bird was found in the birds of group II when autopsied four weeks after inoculation; two of these birds had no worms. On February 24, the remaining birds of group III and each of group IV were fed 4,100 eggs and larvae. Fourteen days later both of these groups were autopsied. The birds of group III harbored a total of 8 pairs of worms of the original infection and 45 pairs of worms of the second infection. However, the 45 pairs of worms of the second infection were found in one bird, which also harbored three of the eight pairs of the original infection. Therefore, it may be readily assumed that some factor or factors must have entered in to break down the resistance or immunity in this bird, since none of the other birds harbored worms of the second infection. This same bird likewise harbored the largest number of pairs of worms of the first infection of any single bird. Twelve of the 22 control birds were infected and none harbored more than 21 pairs of worms.

#### DISCUSSION

An analysis of the data of the foregoing experiments has revealed some interesting facts in addition to those which have a direct bearing on the problem concerned in this paper. It affords, first of all, an opportunity to compare infections in chickens and turkeys of the same age to which were fed similar dosages of infectious material. Each of the birds of groups III of experiments II and IV received an initial dose of approximately 2,500 eggs and larvae. Four weeks later, the surviving birds of each of these groups were each given a second dose of approximately 5,000 eggs and larvae. At necropsy 14 days later, the turkeys were found to have retained more worms of both the first and second infections than did the chickens, thus indicating that the turkey is a more suitable host for this parasite than is the chicken.

The data from group I of experiments II and IV afford another opportunity to compare infections in chickens with those in turkeys. These two groups were tested concurrently and eggs and larvae from the same cultures were used to infect both groups. Both were necropsied 14 days after infection. The results indicate that the turkeys were more susceptible to infection with gapeworms since they harbored three times as many worms as the chickens.

The data from group III of experiments II, III and IV offer a basis for com-

paring the fate of *S. trachea* adults in the three species of hosts when the birds were held for 6 weeks after infection and then autopsied. At that time 1.2 per cent of the worms introduced were recovered from the turkeys, none from the pheasants, and 0.02 per cent from the chickens. It appears, therefore, that pheasants tend to lose their infections more rapidly than turkeys.

It is of particular interest to note also that in every case where comparison could be made, the percentage development of the worms varied inversely with the number of infective eggs and larvae fed. Those groups which involved birds of the same species of about the same age and which were necropsied at about the same time after infections were considered comparable groups. For instance, group III of experiment I and group III of experiment II, both involving turkeys, are comparable. The former group was fed 10,000 eggs and larvae per bird and the latter, 2,500 eggs and larvae per bird. At autopsy, those birds to which the larger doses had been given had fewer than half as many worms as those which had received the smaller doses. Data for these two groups and for four sets of comparable groups involving chickens are presented in Table 2. The differences in percentage development in the groups

TABLE 2.—Data from five comparable sets of birds to show relation between the percentage development of *Syngamus trachea* and the number of eggs and larvae administered

Experiment number	Group number	Number of eggs and larvae fed	Age of bird when fed	No. of days worms were in bird	Percentage development	No. of worms per bird
I	III	10,000	49	37	0.5	22.6
II	III	2,500	41	42	1.2	15.0
V	I	2,100	21	14	1.0	10.1
VI	I	1,500	22	15	1.4	10.8
IV	I	2,500	45	14	0.6	7.6
V	IV	3,000	50	12	0.4	5.4
VI	IV	4,100	50	14	0.2	3.3
V	II	2,100	21	28	0.09	0.9
VI	II	1,500	22	28	0.7	5.6
V	III	2,100	21	41	0.04	0.5
VI	III	1,500	22	42	0.08	0.6

selected from the chicken experiments are smaller than the differences between the groups from the turkey experiments. It must be borne in mind that in all of the examples from the chicken experiments, the differences in the numbers of eggs and larvae fed were comparatively small. Therefore, large differences in percentage development should not be expected.

The cause for such a relationship, as described in this paper, between the amount of infective material fed and the percentage development can only be surmised. A plausible explanation is that the larger dosages of eggs and larvae induced a stronger host reaction than did the smaller dosages. The newly acquired resistance could be directed against the organisms that induced it with the consequent elimination of some or all of them. A smaller initial dosage could induce a milder host response and allow more of the worms to survive. There is no evidence that crowding should be considered as a factor influencing the results.

A similar relationship between the number of eggs or larvae fed and the percentage development has been noted by other workers. Ackert, Graham, Nolf and Porter (1931), working with *Ascaridia galli*, fed initial doses of 500, 100, 50 and 25 eggs to chickens of the same age. Their results indicated clearly that the smaller the

number of eggs fed the larger was the percentage survival. They stated that the cause of this relationship was problematical, but suggested that it might have a serological basis. Winfield (1933), working with *Heterakis spumosa* in rats, fed graded doses of from 50 to 5,000 eggs and found that there was a consistent inverse relationship between the number of eggs fed and the percentage development. He indicated that the protective reaction of the host apparently caused the reduction in the number of worms. Sheldon (1937), working with *Strongyloides ratti*, observed that "the percentage development progressively decreased with increasingly larger doses up to 12,000 larvae. A dose of 14,000 larvae was followed by death and a dose of 15,500 larvae by a very high percentage of development. Doses of from 350 to 8,000 larvae yielded gradient numbers of worms which when plotted rise in an almost straight line. Doses of 10,000 and 12,000 larvae, instead of increasing the worm burden, actually produced markedly fewer worms. Doses of 14,000 and 15,500 appeared to cause a breakdown in the host-protective mechanism. . . ." He stated that "the marked reduction in the number of worms recovered following infection with 10,000 and 12,000 larvae is perhaps explainable on the basis of a rapidly developing acquired resistance."

The results of the experiments reported in this paper indicate that the gapeworms of the initial infection were lost rapidly, so that between the sixth and eighth weeks after being introduced only a relatively small proportion of the worms which had survived the first weeks remained. In experiment I there was an average of 22.6 pairs of worms per bird 37 days after infection, whereas 19 days later there was only an average of 2.3 pairs per bird. In experiment III, the pheasants lost almost all of their worms within 41 days after infection, although an examination of a number of these birds a few days after the worms had reached the trachea revealed that most of the birds carried heavy infections. The results of the experiments with chickens show that they lost their worms rapidly between the 14th or 15th day and the 28th day after infection. This loss in worms may be partly attributed to the development of age resistance, but acquired resistance must also have been a contributing factor. Investigations by Wehr (1939) have shown that gapeworms may survive in the turkey for 224 days and in chickens for 147 days. This would indicate that the loss of worms within a few weeks after infection cannot be attributed to the shortness of the worm's life span. It is rather a reflection of the development of an effective resistance by the host.

#### SUMMARY

Evidence is presented to show that turkeys, ring-necked pheasants and chickens developed marked resistance to infection with the gapeworm, *Syngamus trachea*, as a result of single infections. The resistance in turkeys was tested while worms of the immunizing infection were still in the trachea. Results of the pheasant and chicken experiments suggest that in these host species the resistance may persist after the immunizing infection has been lost.

Turkeys and pheasants of the same age were about equally susceptible to infection. However, the pheasants lost their infection more quickly than did the turkeys. On the other hand, even when very young, chickens were more resistant than either of the other host species.

In every case where comparison could be made the percentage development varied inversely with the number of eggs and larvae administered. This is assumed to



indicate that the larger doses induced a more rapid or a stronger response on the part of the host with the consequent elimination of some or all of the worms. Smaller doses induced a milder reaction and consequently a greater proportion of the worms were able to survive.

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## THE GENUS *AMBLYOMMA* (IXODIDAE) IN THE UNITED STATES\*

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### INTRODUCTION

The publication of a review of the genus *Amblyomma* in the United States has become desirable because of the increasing interest in the members of this genus as known or suspected carriers of diseases and especially because of the need for criteria for the specific differentiation of the larvae and nymphs.

This genus, which is far richer in species than any other, is geographically limited to that portion of the world which is between the 40th parallels of latitude, that is, to the warmer countries. In the United States it is essentially limited to the Southeastern and Southcentral States.

C. L. Koch (1844) was the first to publish a comprehensive account of the ticks. He described many new species and the genus *Amblyomma*. Though he laid the foundation for the systematic classification of ticks, he failed in many instances to select adequate characters. G. Neumann in 1896 began his *Révision de la Famille des Ixodes* which included *Amblyomma* and he was the first to use satisfactory morphological characters and thereby made a definite contribution to the classification of genera and species.

Dönitz (1909) urged the importance of the ornamentation as an aid in recognizing the species and his plan of giving names to the constituent spots and stripes brought order into the terminology of the ornate pattern which most species of *Amblyomma* display. However, the species of *Amblyomma* in the United States are readily separated by morphological characters, hence the ornate color patterns are not described in this paper. They are shown in the figures.

L. E. Robinson (1926) gives excellent descriptions and figures of the 86 species then known and also summarizes their geographical distribution, hosts, economic aspects, and relation to disease.

The more important later writings include Sharif (1928), Dunn (1934), Aragão (1936) and Osorno-Mesa (1941). Other contributions have been made by Warburton (1927), Schulze (1932, 1933, 1936), Kishida (1935) and Cooley and Kohls (1942).

The collection of the Rocky Mountain Laboratory now contains 65 described species of *Amblyomma* from various parts of the world. A study of these and of other available species has led to a fuller understanding of critical morphological and color characters as well as of variation. Variation occurs, particularly in the color patterns, but is less confusing than in some other genera.

A single male specimen of *Amblyomma ovale* (Koch, 1844) taken from a dog, Sept. 8, 1941, Tama Indian Reservation, west of Tama, Iowa, was sent to us for

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identification. *A. ovale* is known from Mexico and South America. The finding of this one specimen in Iowa naturally raised the question of whether this species is indigenous in that State. Concerning this question, Mr. Eddy, the sender, has written us the following:

Relative to the establishment of *A. ovale* in Iowa, I might say that we have no further records. A special attempt was made to collect in the area where the tick was picked up and also in other near by areas. Considering the number of animals that were examined throughout the spring, summer and fall of 1941 and the fact that such animals were taken from this same area, I personally feel that the tick is not established on the Tama Iowa Indian Reservation. I realize that certain animals not checked could serve as normal hosts for this tick in North America, but I feel justified in believing otherwise.

The present paper is based largely upon miscellaneous collections made by members of the staff of the Rocky Mountain Laboratory. The authors wish to express their appreciation to these staff associates and to numerous other professional workers and various institutions in this country that have sent us materials; especially Dr. E. A. Chapin, of the U. S. National Museum; Dr. J. C. Bequaert, of Harvard University; Dr. F. C. Bishopp, of the Bureau of Entomology and Plant Quarantine; to Dr. Paul L. Piercy, of the Agricultural and Mechanical College of Texas; and to Dr. R. R. Parker, Director of the Rocky Mountain Laboratory, for advice and for reviewing this manuscript.

#### THE MEDICAL AND VETERINARY IMPORTANCE OF *Amblyomma* IN THE UNITED STATES

Maver (1911) reported the experimental transmission of the Rocky Mountain spotted fever rickettsia by *Amblyomma americanum* nymphs and adults which as larvae had engorged on an infected guinea pig.

Parker, Philip, and Jellison (1933) demonstrated the survival of this rickettsia from infected female ticks to the larvae of the next generation and transmission by the latter. They considered that the experimental data and the habits and host relationships of *A. americanum* strongly suggested that it was a natural vector of spotted fever. Several instances of possible transmission by this tick in Virginia, Missouri and Louisiana were cited.

In an unpublished paper presented before the American Epidemiological Society on March 20, 1942, Hassler, Sizemore and Robinson discussed seven cases of spotted fever which occurred in 1941 within a period of 32 days in an Oklahoma family in which the circumstantial evidence pointed to *A. americanum* as the vector.

Anigstein (1942) reported four Texas cases in which circumstantial evidence again pointed to *A. americanum* as the vector.

Parker, Kohls, and Steinhaus (1943) reported the recovery of the spotted fever rickettsia from nymphal *A. americanum* collected in Oklahoma. This demonstration of spontaneously infected ticks in nature, together with the accumulated circumstantial case evidence, has definitely established this tick as the third proved vector of spotted fever to man in this country.

*A. americanum* is certainly a potential transmitting agent of tularemia and very possibly is an actual one. However, naturally infected ticks have not been found. In unreported experiments of the Rocky Mountain Laboratory the survival of *Pasteurella tularensis* from the larval to the adult tick was shown (Parker, Philip, Davis and Cooley, 1937).

While no case of American Q fever definitely diagnosed as such has been reported following tick bite, the infectious agent, *Rickettsia diaporica*, has been recovered from several species of ticks. Parker and Kohls (1943) have recorded the recovery of ten strains from *A. americanum* collected in Liberty County, Texas, in 1937.

An apparently new clinical entity designated as Bullis fever has recently been described by Woodland, McDowell and Richards (1943) as occurring in 1942 and 1943 among troops stationed at Camp Bullis, Texas. Epidemiological data suggest that the disease is transmitted by a blood-sucking parasite and that the most likely vector is *A. americanum*.

Parker, Kohls, Cox and Davis (1939) have reported the recovery of a rickettsia pathogenic for guinea pigs from *Amblyomma maculatum* collected in Liberty County, Texas. This rickettsia belongs to the Rocky Mountain spotted fever group and is most closely related to that of boutonneuse fever. This rickettsia is apparently wide spread in the *A. maculatum* population in the South and since its initial isolation has been recovered several times from ticks collected in Texas and also from ticks collected in Georgia. The disease which it produces in guinea pigs is relatively mild. Its pathogenicity for man is unknown. If human infection is caused, it is likely of infrequent occurrence since this tick only occasionally attaches to man.

Woodland, McDowell and Richards (1943) suggested that *Amblyomma americanum* is the vector of an apparently new disease entity designated as Bullis fever, which was prevalent in 1942 and 1943 among troops stationed at Camp Bullis, Texas. Anigstein and Bader (1943a, b) have reported the recovery of a possible rickettsia, which they believe to be associated with this disease from *A. americanum* collected at this same camp.

While *A. cajennense* is a carrier of Brazilian spotted fever of Brazil and of Tobia petechial fever of Colombia, disease conditions which are presumably identical to each other and to Rocky Mountain spotted fever, it has never been found naturally infected with the spotted fever rickettsia in the United States, nor has it been suspected of being a vector. However, it is certainly a potential one. Fortunately, its distribution in this country, as at present known, is limited to the southern tip of Texas, and it appears unlikely to extend its range consequentially.

Because of their very long hypostomes, *A. cajennense* and *A. americanum*, the larvae, nymphs and adults of which all attack man freely, are particularly annoying pests in sections of the country in which they are abundant. When they are removed by pulling, the hypostome is usually left in the skin and is not pulled out as ordinarily happens with our species of *Dermacentor*. The bites of *A. americanum* have been found particularly annoying during the past two years by troops training at some of the Army camps in the South Central States, and in those where this tick has been particularly abundant morale has been noticeably affected.

The following quotation from a letter received from Mr. E. B. Palmer, of Seattle, offers first-hand evidence of the degree of annoyance that these ticks can cause. In this instance the species concerned is almost certainly *A. cajennense*.

I am going to Mexico on November 1st and expect to be there some ninety days hunting jaguar and ocelot. This will be my third trip. The first trip was down near Tampico, the next near Mazatlan and this trip will be near San Ignacio. On both previous trips I suffered the tortures of the damned with the so-called "ticks." Was unable to get its name but the Mexicans call them ticks. . . . On both my trips I was completely covered with them, particularly on my arms and legs. This little fellow is so small that you do not feel him crawl. Where he bites and



after he fills himself with blood the place where he sucked the blood forms a watery blister about the size of a pin-head, and itches so that you have to scratch it. . . . My legs and arms were raw from these bites and my scratching them. I have found that alcohol rubbed on them relieves the itching very materially. The Mexicans who accompanied me on these trips would take dry palm leaves and set them afire and singe themselves much the same as we do chickens or turkeys. They would do this once a day or more. . . . It would be a Godsend to all people hunting in Mexico if they could be relieved from the itching of these ticks.

In the United States the *Amblyomma* are not known to be important as transmitting agents of disease among domestic animals. It is likely that *A. americanum* transmits spotted fever and possibly tularemia to dogs and sheep, but in general such transmission would be of most consequence as a factor in the natural maintenance of the disease agents concerned. However, serious illnesses as a result of tularemia infections are quite possible. These ticks are most important in relation to domestic animals because of debilitation caused by massive infestations. *A. maculatum* is particularly important because "it creates conditions in ears of domestic animals which induce screwworm infestations." (Hixson, 1940.) It is likely that *A. americanum* also functions in the same manner to some extent. Hooker, Bishopp and Wood (1912) wrote:

In the sections where this tick (*A. maculatum*) occurs in any numbers it is the source of great annoyance to domestic animals, particularly to cattle. By attaching to the inside of the ears, as frequently occurs, great irritation is caused; . . . The injury in the ears furnishes opportunity for the screw-worm fly (*Chrysomya macellaria*) to deposit its eggs, which in the case of equines sometimes results in the destruction of the cartilage, thus causing the ears to droop—a condition known as "gotched" ears. The species is also of some importance on account of the fact that it sometimes attacks man.

Robinson (1926), writing of *Amblyomma* in general, stated:

Leaving out of account the question of disease transmission in the strict sense, many of the species of the genus *Amblyomma* cause severe injury to domestic stock—bovines, equines, sheep and goats—by the enormous quantities of blood which they abstract from their hosts when present in large numbers, and by the suppuration of the wounds which they inflict, and by general tick worry.

The spontaneous bacterial flora of *A. americanum* has been found by Dr. Edward A. Steinhaus, of the Rocky Mountain Laboratory, to consist of species of the genera *Bacillus*, *Micrococcus*, *Proteus*, *Alcaligenes* and *Eberthella* (unpublished data).

#### TERMS

*Apical ventral spur*.—The ventral spur at the distal end of the tarsi of adults and rarely in the nymphs of *Amblyomma*. It may be present or absent. Other more proximal ventral spurs may be present.

*Basis capituli*.—(Sometimes abbreviated to "basis.") The basal portion of the capitulum, spoken of as the "basal ring" by some authors. The basis capituli is movably attached to the anterior part of the scutum and lies partly within the emargination.

*Capitulum*.—The anterior portion of a tick which bears the mouth parts, sometimes spoken of as the "false head," "head" or "rostrum." It consists of the basis capituli, the palpi, hypostome, and chelicerae.

*Cornua*.—In *Amblyomma* this useful term is confusing because of the shape and contour of the basis capituli. Cornu (pl. cornua) as herein used means the more or less projecting postero-lateral corner of the basis.

*Coxal spurs.*—Retrograde processes on the coxae. Internal spurs are on the side toward the median line of the tick and external spurs are on the outer side toward the margin of the body.

*Festoons.*—Uniform, more or less rectangular areas along the posterior border of the dorsum, separated by grooves. Present in both sexes.

*Files.*—The longitudinal rows of the denticles or "teeth"—in this paper sometimes referred to as lateral files and median files. The lateral files indicate the outer paired rows. The median files include all except the laterals. Files number 1 are the same as the the laterals, the highest numbered files are those nearest the middle line.

*Frame.*—The elevated chitinous periphery of the spiracular plate. When the "thickness" of the frame is described, reference is made to the transverse thickness.

*Hypostome.*—The ventral mouth part with recurved teeth or denticles arranged in longitudinal rows or files. The length of the hypostome is the measurement from the base to the tip and "about" is used because there is usually no definite point or line at the base from which to measure. The denticles are expressed in a formula as 3/3 or 4/4, indicating the number of files on each half of the hypostome. The denticles may be long and sharp or short and rounded apically. The term "over hang" is sometimes used to indicate the extension beyond the base of the tooth where it is attached.

*Lateral groove.*—In males, the groove at the sides of the scutum which may be complete, incomplete, or absent.

*Legs.*—Measurements of the length of articles are made from the dorsal side rather than from the ventral side which "telescopes."

*Macula.*—In adult ticks, the more heavily sclerotized spot on the spiracular plate through which the principal respiratory opening passes.

*Marginal groove.*—In females the groove at the sides of the postscutal area of the body; present in unfed ticks and disappearing as the female becomes distended with feeding.

*Millimeters.*—All measurements are in millimeters expressed in numerals without repeating the usual "mm."

*Nubs.*—Small projections on the posterior edge of the ventral scute.

*Ornate.*—Adults and nymphs of *Amblyomma* are either ornate or inornate, which refers to the presence or absence of a pattern of colors which include dark and light units. The pattern may be in connected lines and broad areas or in separated spots or with both. Emphasis is on the pattern rather than on the color, which varies somewhat. While an extended terminology of the members of the pattern was erected by Dönitz (1909) and revised by Robinson (1926), such terminology is omitted in this paper because it is not needed in distinguishing between the United States species. The reader is referred to the particular figures in which the pattern is shown.

*Salience.*—A definite edge which projects more or less.

*Trochantal spur.*—Spur on the ventral surface of the trochanter, similar to coxal spurs.

*Ventral plaques.*—More or less definite sclerotized plates situated just anterior to the ventral scutes. Present in most males of *Amblyomma* but of little use in differentiating United States species.

*Ventral scutes.*—Chitinous thickenings of the ventral surface of the festoons which may be very distinct and protruding, faint or absent. They may also bear nubs.

GENUS *Amblyomma* C. L. KOCH 1844

1844. *Amblyomma* Koch, p. 223.  
 1872. *Adenopleura* Macalister, p. 287.  
 1877. *Xiphiastor* Murray, p. 201.  
 1899. *Amblyomma* Koch: Neumann, p. 200.  
 1908. *Amblyomma* Koch: Banks, p. 37.  
 1911. *Amblyomma* Koch: Neumann, p. 53.  
 1926. *Amblyomma* Koch: Robinson, p. 9.

Usually ornate with dark spots and stripes on a pale background. Eyes and festoons present. Palpus usually long with article 2 especially long. Basis capituli of variable form, often sub-quadrangular or sub-triangular. Males without the adanal shields found in *Rhipicephalus*, *Boophilus* and *Hyalomma* though they may have ventral plaques or ventral scutes which may be extended beyond the margin of the body. Spiracular plates sub-triangular or comma-shaped. Nymphs resemble adults but may differ in the shape of the spiracular plate and are seldom ornate.

Genotype: *Amblyomma cajennense* (Fabricius, 1787.)

## Key to Females

		PAGE
1. Scutum, inornate	<i>inornatum</i>	105
Scutum, ornate	2	
2. Coxa I with external spur distinctly longer than the internal spur	3	
Coxa I with subequal spurs	5	
3. Scutum with the pale markings usually limited to a spot near the posterior end	<i>americanum</i>	87
Scutum with pale markings in an extensive pattern	4	
4. Coxa I with internal spur about half the length of the external spur	<i>cajennense</i>	83
Coxa I with the internal spur very short or insignificant	<i>maculatum</i>	94
5. Coxa IV with the external spur longer than the internal spur	<i>dissimile</i>	99
Coxa IV with the two spurs about equal	<i>tuberculatum</i>	102

## Key to Males

		PAGE
1. Scutum, inornate	<i>inornatum</i>	106
Scutum, ornate	2	
2. Coxa I with the internal spur moderately long	3	
Coxa I with internal spur short or insignificant	4	
3. Scutum with the pale markings in an extensive, connected pattern	<i>cajennense</i>	84
Scutum with the few pale markings in isolated spots	<i>americanum</i>	88
4. Coxae II, III, and IV each with one spur	<i>maculatum</i>	95
Coxae II, III, and IV each with two spurs	5	
5. Coxa IV with the external spur distinctly longer than the internal spur	<i>dissimile</i>	100
Coxa IV with both spurs short	<i>tuberculatum</i>	102

## Key to Nymphs

		PAGE
1. Scutum, ornate .....	<i>tuberculatum</i>	103
Scutum, inornate .....	2	
2. Basis capituli pointed at the sides .....	<i>maculatum</i>	95
Basis not pointed at the sides .....	3	
3. Hypostome with dentition 3/3 .....	<i>dissimile</i>	102
Hypostome with dentition 2/2 .....	4	
4. Scutum with conspicuous punctations and with surface pebbled .....	<i>avicolens</i>	107
Scutum with moderate punctation or with none and with surface smooth .....	5	
5. Scutum with punctations distinct, deep, grooves shorter and a little wider .....	<i>americanum</i>	88
Scutum with punctation faint, shallow, grooves longer, and a little narrower .....	<i>cajennense</i>	84

The nymphs are easily separated except those of *americanum* and *cajennense*. Nymphs of *inornatum* have not been seen.

*Amblyomma cajennense* (Fabricius, 1787)

(Figs. 1 and 2)

1787. *Acarus cajennensis* Fabricius, p. 372.  
 1794. *Ixodes cajennensis* (Fabricius), p. 427.  
 1805. *Ixodes cajennensis* (Fabricius), p. 354.  
 1821. *Ixodes crenatus* Say, II, p. 76.  
 1844. *Amblyomma cajennense* (Fabricius) : Koch, p. 226.  
 1844. *Amblyomma tenellum* Koch, p. 227.  
 1844. *Amblyomma mixtum* Koch, p. 227.  
 1887 (1884?). *Ixodes herrerae* Duges, p. 487.  
 1888. *Amblyomma sculptum* Berlese, p. 192.  
 1899. *Amblyomma parviscutatum* Neumann, p. 208.  
 1899. *Amblyomma cajennense* (Fabricius) : Neumann, p. 205.  
 1907. *Amblyomma cajennense* (Fabricius) : Hunter and Hooker, p. 60.  
 1908. *Amblyomma versicolor* Nuttall and Warburton, p. 407.  
 1912. *Amblyomma cajennense* (Fabricius) : Hooker, Bishopp and Wood, p. 151.  
 1926. *Amblyomma cajennense* (Fabricius) : Robinson, p. 288.

## Female

*Body*: Length of unengorged specimens from 3.12 to 3.42, width from 2.34 to 2.55. Oval, with the width over length varying considerably. Scutum extending about half the length. Marginal groove complete and continuing across the basis of the festoons. Nubs on the ventral scutes sometimes visible from above. Well engorged females may become as large as 13.50 × 11.00 and are about equally broad on both ends.

*Capitulum*: Length from 1.11 to 1.5; width of basis, 0.66. Basis sub-rectangular and with very mild cornua. Porose areas small, nearly circular. Surface smooth, shining, punctate. Palpi long, compressed laterally. Hairs moderate in number.

*Scutum*: Length 1.62 to 1.86, width 1.71 to 1.86. Triangular, usually a little broader than long, sometimes with length and breadth equal, widest in front of the middle, postero-lateral margins nearly straight. Cervical grooves short, deep in front, then continuing posteriorly as shallow valleys. Scapulae narrow. Eyes moderate in size, pale and very mildly convex. Ornate with whitish pattern variable. Punctations moderate in number, usually larger in the antero-lateral areas.

*Legs*: Moderate in length and size, relatively slightly longer and smaller than in the male. Distal end of tarsus I with apical and sub-apical ventral spurs absent, present on II, III and IV. Length of tarsus I, 1.05; metatarsus, 0.75. Length of tarsus IV, 0.66; metatarsus, 0.75.



*Coxae*: Coxa I with two distinct spurs, the external spur much longer. Coxae II, III and IV each with a single, broad, flat, rounded spur. All coxae with a few, long, fine hairs.

*Spiracular plate*: Frame moderately well sclerotized, surface a little concave at the macula. Length 0.51, width 0.51.

*Genital aperture*: Situated at about the level of the intervals between coxae II and III.

*Hypostome*: Spatulate, long, notched apically. Dentition 3/3. Length 0.93.

#### Male

*Body*: Length from 2.34 to 3.12, width 1.80 to 2.40. Robinson (1926) gives the length as 3.9 to 4.9 and width 2.3 to 3.0. Broad oval, wider behind.

*Scutum*: Smooth, shining, and with the dark-colored spots slightly elevated over the light-colored ones; scapulae pointed. Cervical grooves short and deep. Lateral grooves distinct posteriorly and across the festoons, fading out back of the eyes. Conspicuous extensions of the ventral plaques present in some specimens. Eyes small, and slightly elevated. Ornamented, with a characteristic pattern which includes spots on some of the festoons. Punctations moderate in size and number, the larger ones limited mainly to the light-colored areas.

*Capitulum*: Length from 0.84 to 1.05, width of basis from 0.48 to 0.66. Basis subquadrate, sides a little convex, cornua short to moderate. Surface smooth, shining, punctate. Palpi long, compressed laterally. Combined length of I and II, 0.51 to 0.54.

*Hypostome*: Spatulate, long, apically rounded and very faintly notched. Dentition 3/3. Length about 0.72.

*Legs*: Moderate in length, with their sizes progressively increasing from I to IV. Terminal and sub-terminal ventral spurs absent on tarsus I; present on II, III and IV. Length of tarsus I, 0.87; metatarsus, 0.66. Length of tarsus IV, 0.60; metatarsus, 0.78.

*Coxae*: Coxa I with two long spurs, the external one longer. Coxae II and III each with one short, broad, flat spur. Coxa IV with one long, internal spur.

*Spiracular plate*: Large, moderately well sclerotized, with the surface slightly concave. Length 0.66, width 0.36.

*Genital aperture*: Situated at about the level of the intervals between coxae II and III.

#### Nymph

*Body*: Broad oval. Length (unengorged) 1.38, width 1.14.

*Capitulum*: Length 0.42, width of basis 0.3. Basis sub-quadrate, sides convex, cornua faint or absent, posterior margin concave or nearly straight. Surface smooth, impunctate. Palpi long and with a few fine hairs. Combined length of articles 2 and 3, 0.24.

*Hypostome*: Spatulate, moderately long, faintly notched apically. Dentition 2/2. Length about 0.24.

*Scutum*: Length 0.66; width 0.84. Broadly cordiform, wider than long, broadest at about the middle, scapulae rounded. Cervical grooves deep and long, reaching to near the postero-lateral margins. Eyes large, pale, mildly convex. Punctations moderate in number, small, faint, seen best in reflected light.

*Legs*: Ventral spurs absent on distal ends of all tarsi. Length of tarsus I, 0.42; metatarsus, 0.24. Length of tarsus IV, 0.30; metatarsus, 0.21.

*Coxae*: Coxa I with two distinct spurs, the internal one shorter. Coxae II, III and IV each with a single spur. All spurs short, those on II, III and IV flat and relatively a little narrower than in *americanum*.

*Spiracular plate*: Large, nearly flat, but sunken at the respiratory opening. Length 0.21, width 0.14.

#### Larva

*Scutum*: Length 0.235, width 0.336. Much wider than long. Cervical grooves shallow, long, and about parallel. Surface smooth, faintly shagreened, impunctate.

*Capitulum*: Length 0.18, width 0.15. Basis short and broad, with rounded points at the sides. Surface smooth, shining, impunctate. Palpi long, with articles 2 and 3 about equal, hairs few and fine. Combined length of articles 2 and 3 about 0.11.

*Hypostome*: Short and broad distally. Dentition 2/2. Length about 0.086.

#### DISTRIBUTION AND HOSTS

The type locality of *A. cajennense* is Guiana. Robinson (1926, p. 51) summarized the known distribution of *A. cajennense* and included the previous data of Koch (1844), Stoll (1888-1893), Berlese (1888), Neumann (1899), Banks (1908),

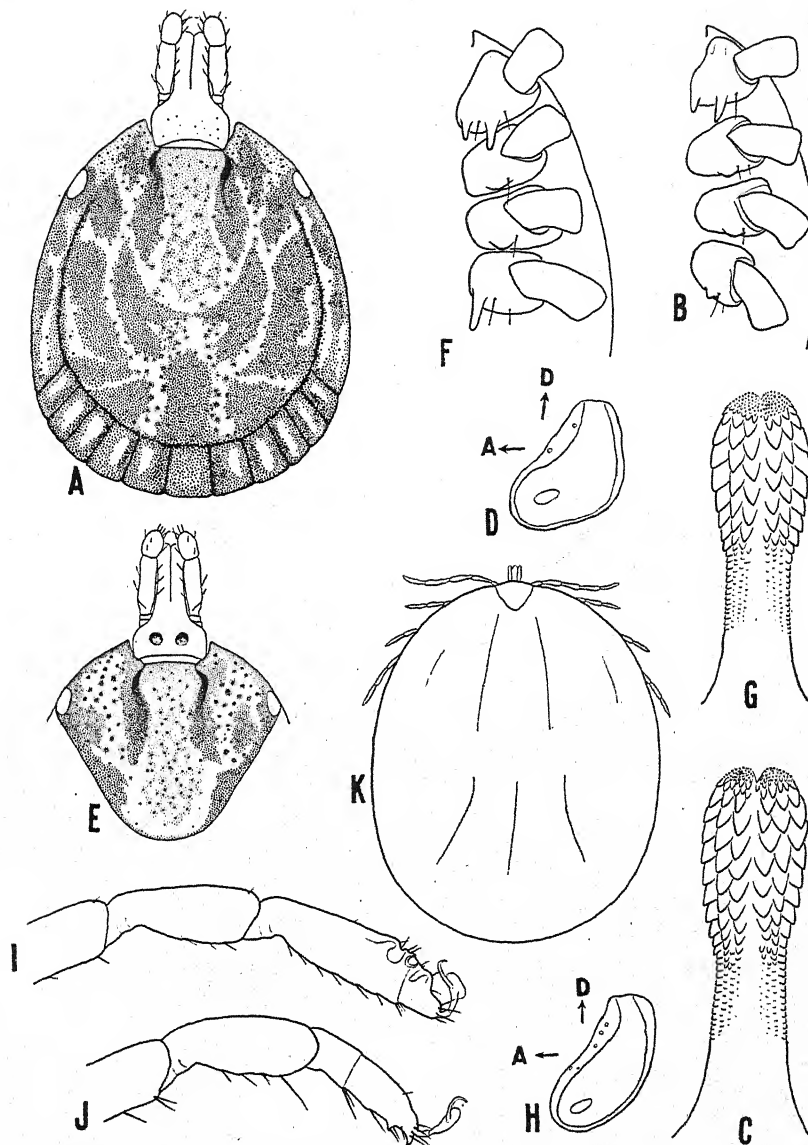


FIG. 1. *Amblyomma cajennense* (Fabricius). A. Capitulum and scutum, male. B. Coxae of female. C. Hypostome, female. D. Spiracular plate, female. E. Capitulum and scutum, female. F. Coxae of male. G. Hypostome, male. H. Spiracular plate, male. I. Leg I, female. J. Leg IV, female. K. Engorged female.

Hunter et al (1907) and Aragão (1911). He reported the species as occurring in Southern United States, Central America, West Indies and South America, particularly along the Atlantic seaboard as far south as Buenos Aires. The following countries other than the United States are definitely mentioned: Mexico, Brazil, Guatemala, Nicaragua, Costa Rica, Colombia, Guiana, Panama, Honduras, Cuba, Trinidad, Vera Cruz, Yucatan, Venezuela, Paraguay and Argentine Republic. Hunter et al (1907) include Bermuda. Banks (1908, p. 42) and Hooker et al (1912, p. 152) indicate this tick to be established in the United States only in the

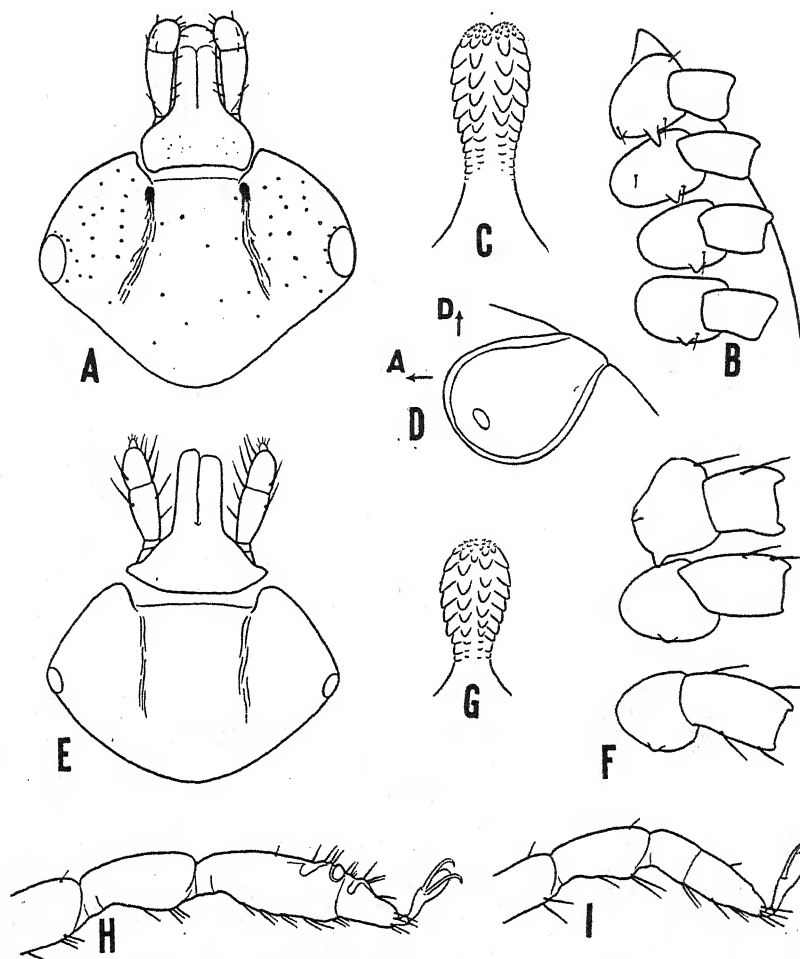


FIG. 2. *Amblyomma cajennense* (Fabricius). A. Capitulum and scutum, nymph. B. Coxae of nymph. C. Hypostome, nymph. D. Spiracular plate, nymph. E. Capitulum and scutum, larva. F. Coxae of larva. G. Hypostome, larva. H. Leg I of nymph. I. Leg IV of nymph.

southern end of Texas and southern point of Florida. References by Banks (1908), and others, and unpublished records of the Rocky Mountain Laboratory show that specimens of this tick have been found in scattered localities in several States. The Laboratory also has specimens from several of the countries listed above, as follows: From Mexico on man, opossum, *Sciurus*, and domestic animals, 17 lots; from Colombia on domestic animals, 8 lots; from Brazil on man, domestic animals, and capybara, 16 lots; from Honduras on domestic animals, 5 lots; from Nicaragua on man and domestic animals, 6 lots; from the Canal Zone, hosts not stated, 4 lots. Including the records of previous writers, some 25 different animals have been reported as hosts.

Dr. Paul L. Piercy has kindly shown us the extensive records of his survey of ticks in Texas and it seems perfectly evident that the tick is abundant and established only in the following counties in Texas: Nueces, Jim Wells, Kleberg, Brooks, Kennedy, Starr, Hidalgo, Willacy and Cameron. From the Banks' record it is apparent that *A. cajennense* was at least formerly present in the southern point of

Florida. However, there have been no records published subsequently of the presence of this species in Florida. In a recent letter from Homer Hixson, he states:

The status of the tick *Amblyomma cajennense* in Florida is apparently not definitely known. Although I have not known of any intensive collecting in Southern Florida, the collecting I have known about indicates that this tick does not occur in Florida or if it does, it is rare.

*A. cajennense* commonly attacks man, horses, cattle, sheep, goats, dogs and pigs. Neumann (1909, p. 442) mentions several authentic records of its occurrence on the tongues of young calves and gives one record on *Bufo marinus*.

Records of the Rocky Mountain Laboratory for the United States are given in the accompanying table.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
10021	Tex.	Brownsville, Cameron Co.	2/8/34	Goat	318 A.	L. Demieville
10048	"	"	4/14/34	?	372 A.	"
10036	"	"	Mar. 1935	.....	23 N., 76 A.	"
11062	"	"	Apr. 1935	Goat	90 A.	"
11060	"	"	4/13/35	Cow	65 A.	"
11061	"	"	"	Horse	105 A.	"
11087	"	"	May 1935	Cow	100 A.	"
11063	"	"	5/11/35	Goat	165 A., 30 N.	"
11119	"	"	June 1935	Cow	10 A.	"
14227	"	"	5/3/38	Dogs	?	R. M. L.
14229	"	"	"	Horses	110 A.	"
14232	"	"	5/4/38	"	100 A.	"
14234	"	"	5/5/38	"	100 A.	"
14235	"	"	"	Drag	125 N., 30 A.	"
20479	"	"	9/14/42	.....	2 A.	St. Bd. Health
11436	"	Kingsville, Kleberg Co.	10/16/35	Peccaries	3 N., 10 A.	R. M. L.
11442	"	"	10/18/35	Drag	20 A.	"
14183	"	"	5/25/38	Deer	3 A.	R. M. L.
14833	"	"	7/25/38	Peccaries	1 A.	J. C. Brown

*Amblyomma americanum* (Linnaeus, 1758)  
(Figs. 3 and 4)

1758. *Acarus americanus* Linnaeus, p. 615. (Not *Acarus americanus*, *A. nigra*, from Brazil, of Treviranus, 1831, pp. 185-191. See Salmon and Stiles 1901, p. 475.)
1778. *Acarus nigra* de Geer, p. 153.
1804. *Ixodes nigra* (de Geer) : Latreille, p. 52.
1804. *Rhynchoprion americanum* (Linnaeus) : Hermann, p. 71.
1805. *Ixodes americanus* (Linnaeus) : Fabricius, p. 356.
1844. *Amblyomma americanum* (Linnaeus) : Koch, p. 229.
1869. *Ixodes unipunctata* Packard, p. 66.
1880. "*Argas americanum* de Geer" of Megnin, p. 134 (in part).
- 1886-1893. *Amblyomma foreli* Stoll, p. 21.
1899. *Amblyomma americanum* (Linnaeus) : Neumann, p. 209.
1901. *Amblyomma americanum* (Linnaeus) : Salmon and Stiles, p. 475.
1908. *Amblyomma americanum* (Linnaeus) : Banks, p. 40.
1912. *Amblyomma americanum* (Linnaeus) : Hooker, Bishopp and Wood, p. 142.
1926. *Amblyomma americanum* (Linnaeus) : Robinson, p. 45.
1929. *Amblyomma americanum* (Linnaeus) : Oudemans, p. 198.
1936. *Amblyomma americanum* (Linnaeus) : Oudemans, p. 455.

A relatively small tick, with a considerable variation in size and also with an unusual disparity between the smaller males and larger females.

Female

*Body*: Length, unengorged, 2.46 to 3.40, width 1.98 to 2.60. Broad oval, with the scutum reaching about half the length. Well engorged specimens may become as large as 11.0×9.25.

*Scutum*: Length 3.30, width 1.80. Sub-triangular, posterior angle broad, posterior apex



flattened, postero-lateral sides nearly straight, scapulae pointed. Posterior angle with a conspicuous whitish spot with red and green tinges; sometimes with other spots in the scapular fields. Punctations numerous, rather evenly distributed and larger in the anterior areas. Cervical grooves short and deep, convergent posteriorly. Eyes flat, large, and pale.

*Capitulum*: Length 0.90 to 1.14, width of basis 0.60 to 0.73. Basis sub-rectangular, dorsal surface smooth, punctate, convex, with sides more declivitous; posterior margin nearly straight, salient; postero-lateral corners rounded, mildly suggesting cornua. Porose areas oval, divergent anteriorly, and separated by about the length of the longer axis of one. Palpi long, with the surface irregular. Combined length of 2 and 3, 0.84.

*Hypostome*: Long, spatulate, faintly notched apically; denticles 3/3. Length about 0.75.

*Legs*: Long and small and with long, fine hairs. Apical and sub-apical ventral spurs absent on tarsus I, present on II, III and IV. Length of tarsus I, 0.96; metatarsus, 0.66. Length of tarsus IV, 0.75; metatarsus, 0.70.

*Coxae*: Coxa I with two spurs, the external one long and pointed, a single broad, plate-like spur each on II and III, a single, triangular, plate-like spur on IV. Long, fine hairs on all coxae.

*Spiracular plate*: Large and with its frame moderately sclerotized, surface concave. Length 0.60, width 0.54.

*Genital aperture*: Situated at the level of the intervals between coxae II and III.

#### Male

*Body*: Length 2.95, width 2.26. Oval, wider behind.

*Scutum*: Smooth and shining. Surface in general flattened, more convex in front of the festoons. Cervical grooves short, deep, and convergent anteriorly. Lateral grooves distinct near the festoons, disappearing back of the eyes. Festoons long, all limited anteriorly by a continuation of the marginal groove. Ornamentation limited to symmetrical, isolated whitish spots as shown in the figure. In some specimens the white spots are much smaller or absent. Punctations numerous and small. Eyes flat, pale.

*Capitulum*: Length 0.9, width of basis 0.59. Basis sub-quadrate, smooth, shining, and punctate; cornua short. Palpi long, with the surface irregular. Combined length of articles 2 and 3, 0.54.

*Hypostome*: Essentially as in the female. Denticles 3/3. Length about 0.48.

*Legs*: Legs long and small, and with long, fine hairs. Apical and sub-apical ventral spurs absent on tarsus I, present on II, III and IV. Length of tarsus I, 0.69; metatarsus, 0.48. Length of tarsus IV, 0.54; metatarsus, 0.54.

*Coxae*: Coxa I with two spurs, the external one longer and pointed. Coxae II and III each with a single broad, flat spur. Coxa IV with a long, pointed, internal spur. All coxae with long, fine hairs.

*Genital aperture*: Situated between coxae II.

*Spiracular plate*: Large, convex, and with the frame only moderately sclerotized. Length 0.51, width 0.28.

#### Nymph

*Body*: Broad oval. Length (unengorged) 1.32, width 1.23.

*Capitulum*: Length 0.42, width of basis 0.30. Basis broad and short. Posterior margin nearly straight, salient. Surface smooth, shining, and with a very few punctations. Palpi long, combined length of 2 and 3, 0.21.

*Hypostome*: Long, rounded apically. Dentition 2/2. Length 0.27.

*Scutum*: Length 0.6, width 0.75. Broadly cordiform, usually slightly broader than long, broadly rounded behind. Cervical grooves deep in front, becoming shallow behind and disappearing back of the eyes. Eyes large, pale, and a little convex. Surface smooth, shagreened. Punctations moderate in number, larger, and more easily seen than in *cajennense*.

*Legs*: Apical and subapical ventral spurs absent on all tarsi. Length of tarsus I, 0.33; metatarsus, 0.21. Length of tarsus IV, 0.28; metatarsus, 0.19.

*Coxae*: Coxa I with both external and internal spurs present, coxae II, III and IV only with the external spur present. All spurs short.

*Spiracular plate*: Large, mildly sclerotized. Length 0.26, width 0.18.

#### Larva

*Capitulum*: Length 0.20, width of basis 0.15. Basis short, broad, and rounded at the sides. Surface smooth, shining and impunctate. Palpi short, laterally compressed, and with relatively long hairs. Length about 0.11.

*Hypostome*: Small, spatulate in shape, rounded apically. Dentition 2/2. Length about 0.66.

*Scutum*: Shape much as in the nymph, but relatively shorter. Cervical grooves moderately deep, long, parallel. Surface smooth, shagreened, impunctate. Length 0.25, width 0.35.

*Coxae*: Coxa I with two small spurs; II and III with one spur.

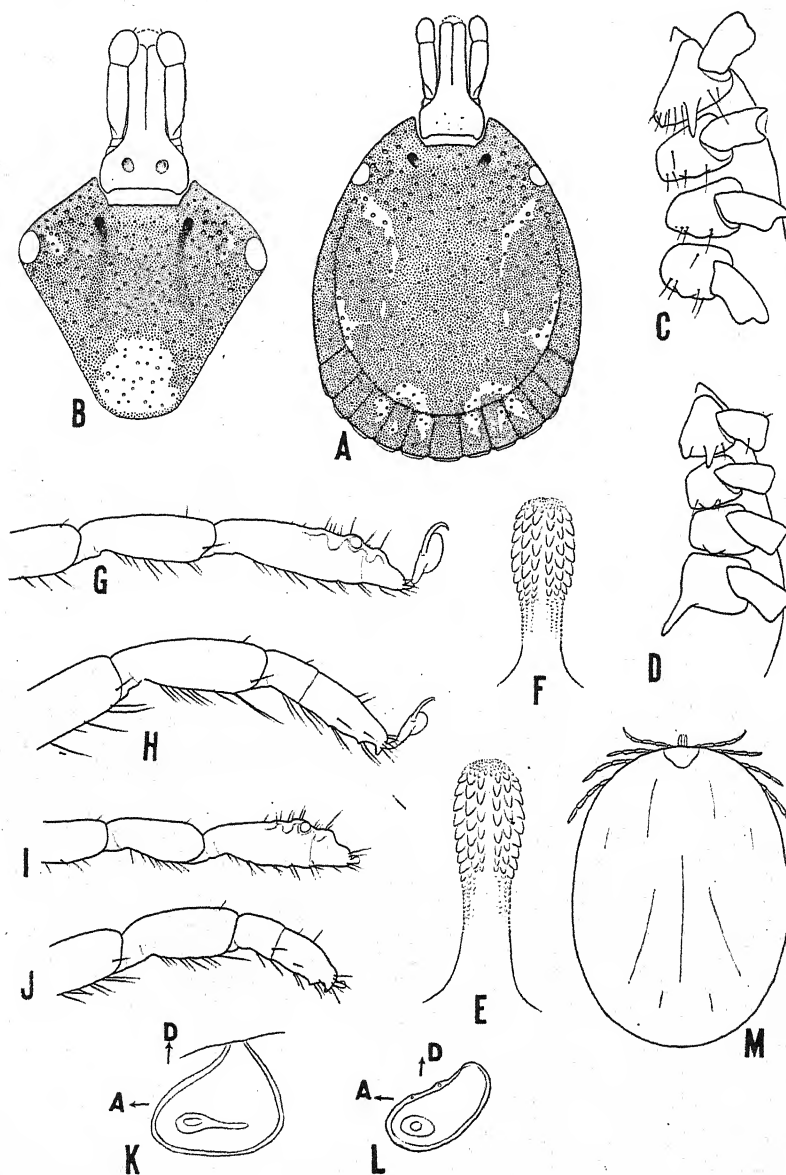


FIG. 3. *Amblyomma americanum* (Linnaeus). A. Capitulum and scutum, male. B. Capitulum and scutum, female. C. Coxae of female. D. Coxae of male. E. Hypostome, female. F. Hypostome, male. G. Leg I, female. H. Leg IV, female. I. Leg I, male. J. Leg IV, male. K. Spiracular plate, female. L. Spiracular plate, male. M. Engorged female.

#### DISTRIBUTION AND HOSTS

The type locality of *americanum* is Pennsylvania or New Jersey but the type host is not known.

Knowledge of the distribution of this tick in the United States is rather indefinite and we can do no better than to quote from a recent publication by Parker, Kohls and Steinhaus (1943), as follows:

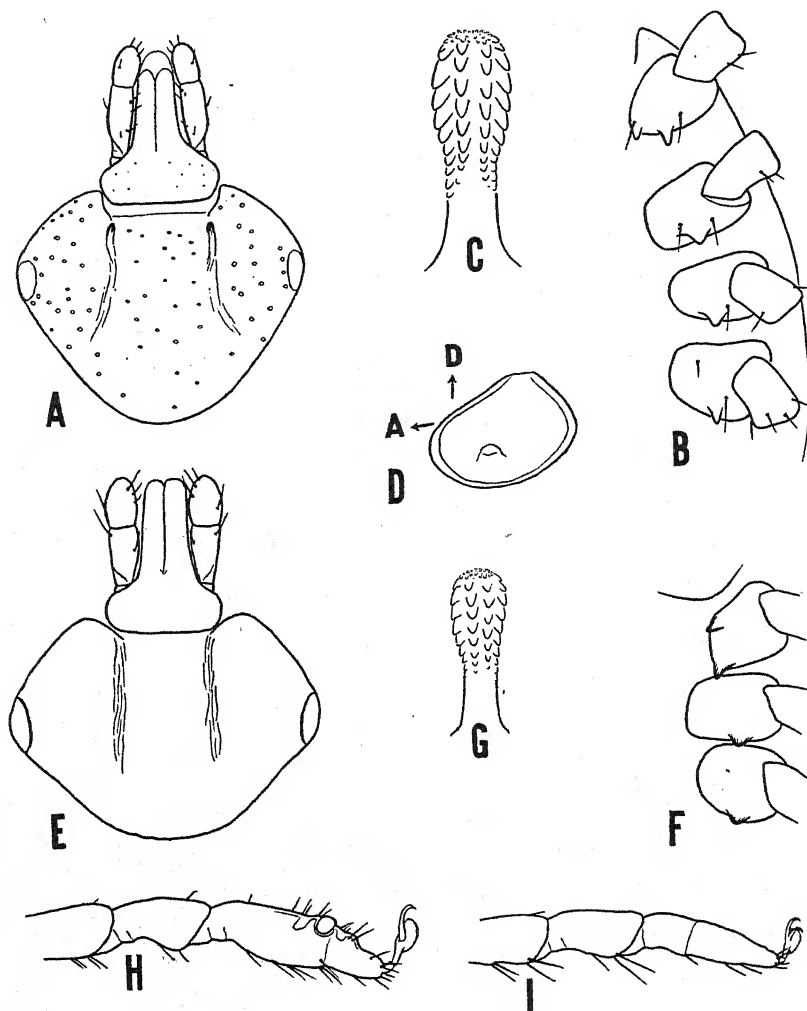


FIG. 4. *Amblyomma americanum* (Linnaeus). A. Nymph, capitulum and scutum. B. Nymph, coxae. C. Nymph, hypostome. D. Nymph, spiracular plate. E. Larva, capitulum and scutum. F. Larva, coxae. G. Larva, hypostome. H. Leg I of nymph. I. Leg IV of nymph.

Hooker, Bishopp and Wood (1912) show *A. americanum* as occurring east and south of a line starting from a short distance west of the southernmost tip of Texas and extending northward and northeastward across the States of Oklahoma, Kansas (southeastern corner), Missouri, Illinois, and Indiana into southern Michigan, and thence almost directly eastward across New York and the southern portions of the three northern New England States. However, such evidence as we have suggests that it is extremely scarce in the northern portion of this area.\* We also have recent reports of its occurrence in southern Iowa. Actually, there is very little published information concerning the distribution and abundance of this tick in any of the 18 States concerned. Apparently it is most abundant in parts of Texas, Louisiana, Oklahoma, Arkansas, and Missouri. Doubtless it is abundant, at least sporadically, in other southern States eastward to the Atlantic Coast.

\*The State Entomologists of Ohio, Indiana, and Illinois (J. S. Houser, J. J. Davis, and W. P. Flint, respectively), and R. E. Rebrassier, of the College of Veterinary Medicine of Ohio State University, have recently informed us that they know of no records of *A. americanum* in their respective States.

The Marx collection contained one specimen from Labrador. Neumann (1911) extended the range of the species to Guatemala, Brazil and Guiana and our records suggest it is common in Mexico.

*A. americanum* shows a very wide range of hosts, including birds. All three stages commonly bite man. Neumann (1911) records it on man, cow and *Felis pardalis*. Hooker et al record it on man, dog, cattle, horse, deer, goat, peccary, hog, mule, skunk, sheep, wolf, fox, squirrel, badger, domestic cat, wild turkey and chaparral cock. The records on dog, cattle, man and horse are most frequent and in that order. Our records add the cotton rat, wild hog (Mexico) and "Tinamou" (Mexico).

Rocky Mountain Laboratory records of this tick in the United States are in the accompanying table.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14191	Ark.	Harrison, Boone Co.	6/6/38	Dogs	104 A.	R. M. L.
14192	"	"	"	Cows	134 A., 1 N.	"
14196	"	"	6/7/38	7 dogs	2 A.	"
14813	"	Alpine, Boone Co.	6/8/38	2 horses, cow	12 A.	"
15506	"	Boone Co.	3/14/39	Dog	1 ♀	S. J. Carpenter
16653	"	Lead Hill, Boone Co.	7/5/39	Dog, cattle, horse	75 ♀, 14 ♂	"
16654	"	Boone Co.	7/7/39	Dog	82 ♀, 26 ♂	"
15520	"	Calhoun Co.	4/4/39	Cow	8 ♀	"
16655	"	Denver, Carroll Co.	7/6/39	Dog	2 N., 7 ♂, 30 ♀	"
15517	"	Sparkman, Dallas Co.	4/3/39	"	14 ♂, 14 ♀	"
15516	"	Sparkman, Dallas Co.	4/3/39	Cow	4 ♀, 2 ♂	"
19047	"	Hot Springs, Garland Co.	.....	.....	36 N., 22 L.	Bur. An. Ind.
20293	"	Ouachita Co.	July '43	.....	1 N., 2 A.	W. J. Baerg
20292	"	Union Co.	.....	.....	1 A.	"
20274	"	Washington Co.	6/20/43	.....	1 A.	"
20291	"	"	7/10-14 1943	Man	1 A.	"
19046	Ga.	Broxton, Coffee Co.	6/4/42	Dog	2 N., 3 A.	Dr. Geo. Brigham
20233	"	Blackbeard Isl. (off coast of Georgia)	7/13/42	Soil	1 A.	Herbert H. Ross
18885	Ia.	Osceola, Clarke Co.	6/28/41	Cow	1 ♀	G. S. Cantonwine
19772	La.	Kisatchie Nat. For., Grant Parish	June 1941	.....	2 ♀	Jones & Archer
7661	Miss.	Phoenix, Yazoo Co.	7/22/31	.....	36 ♀, 21 ♂	.....
13425	"	Vicksburg, Warren Co.	2/26/37	Dog	1 ♂	R. M. L.
20496	"	Harrison Co.	8/20/43	Man	1 N.	F. N. Young, Jr.
14817	Mo.	Golden, Barry Co.	Oct. 1937	Man	1 N.	H. H. Echwalt
14826	"	Cassville, Barry Co.	6/8/38	3 cottontail rabbits	47 N.	R. M. L.
14827	"	Golden, Barry Co.	6/8/38	Timber wolf	5 ♀	"
14822	"	Cassville, Barry Co.	6/9/38	12 dogs	29 A., 16 N.	"
14823	"	"	"	1 dog	14 A.	"
14828	"	"	"	12 dogs	70 N., 11 A.	"
14829	"	"	"	"	40 N., 14 A.	"
14830	"	"	"	5 dogs	47 N., 30 A.	"
14831	"	"	"	"	24 N., 40 A.	"
14824	"	Eagle Rock, Barry Co.	"	"	35 N., 36 A.	"
14825	"	"	"	"	13 N., 32 A.	"
14824	"	Golden, Barry Co.	"	Timber wolf	35 N., 56 A.	.....
14821	"	Viola, Barry Co.	6/10/38	8 cottontail rabbits	41 N.	R. M. L.



Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
16654	"	Bergman, Boone Co.	7/7/39	Dogs	100 A.	"
20276	"	Jefferson City, Cole Co.	6/26/43	Dog	2 A.	Dr. John W. Williams, Jr., St. Bd. Health
19048	"	Hot Springs, Garland Co.	.....	....	2 N.	Dr. H. H. Little
16653	"	Lead Hill, Marion Co.	7/5/39	Dogs	8 A.	R. M. L.
14370	Okla.	Atoka, Atoka Co.	6/6/35	Dog	4 A.	"
19233	"	Durant, Bryan Co.	9/6/41	Dogs and grass	7 N.	Dr. F. R. Hassler
19929	"	Armstrong, Bryan Co.	9/4/42	Drag	180 N.	R. M. L.
19931	"	Wilson, Carter Co.	9/9/42	Cow and dog	18 N.	"
19932	"	"	"	Drag	490 N.	"
20488	"	Cache, Comanche Co.	3/4/42	Coyote	1 A.	Frank B. McMurry
8127	"	Broken Bow, McCurtain Co.	2/20/32	Cow	1 ♀	Mr. Sanborn
20249	"	Camp Gruber, Muskogee Co.	6/1/43	Drag	3 N., 13 A.	Herbert H. Ross
20295	"	"	5/15-6/11/43	Man	14 N., 16 A.	Michael Ballin
20294	"	"	6/11 & 7/1/43	"	11 N., 8 A.	"
19933	"	Weathers, Pittsburg Co.	9/11/42	6 dogs	3 A.	R. M. L.
19934	"	"	"	Drag	110 N.	"
20287	Tenn.	Baird's Mills, Wilson Co.	7/12/43	....	86 A.	War Dept., Nashville
20300	"	"	8/1/43	....	12 N., 6 A.	"
20453	"	"	8/8/43	....	35 A.	"
20451	"	"	8/9/43	....	250 N., 1 A.	"
20457	"	"	8/10-14/43	Ground	354 N.	"
20454	"	"	8/12/43	....	460 N.	"
20460	"	Nashville, Davidson Co.	8/24/43	....	387 N.	"
20462	"	"	8/25/43	....	101 N.	"
20463	"	"	8/27/43	....	225 N.	"
20244	Tex.	Camp Hood, Bell Co.	5/30/43	....	1 N., 4 A.	Dr. David Lackman
20290	"	"	4/26/43	Man	3 A.	"
19881	"	West Columbia, Brazoria Co.	?	....	3 ♀	T. McGregor
19951	"	"	6/23/42	Man	1 N.	Dr. W. Gingrich
19952	"	"	"	"	1 N.	"
19919	"	"	8/22/42	Drag	2500 N.	R. M. L.
19920	"	"	8/24/42	Cow	1 ♀, 1 N.	"
19922	"	"	"	Drag	900 N.	"
19921	"	"	"	Dog	?	"
19923	"	"	8/25/42	Drag	2000 N., 1 ♂	"
20467	"	Brazos Co.	4/19/43	....	4 N.	State Bd. Hlth.
20472	"	Brown Co.	10/10/42	Dog	1 N.	"
17312	"	Colorado Co.	5/30/40	Deer	1 N., 4 A.	T. T. Waddel
20458	"	Dallas Co.	8/5/43	....	19 N.	Southwest Biological Sup. Co.
20464	"	Cuero, DeWitt Co.	5/21/43	....	5 A.	State Bd. Hlth.
13922	"	Tartleton, Prairie, Erath Co.	7/29/37	Cows	22 N.	R. M. L.
13924	"	"	"	"	4 A.	"
13935	"	"	8/3/37	Cow	3 A.	"
13936	"	"	"	Horse	2 N.	"
13931	"	"	8/5/37	Cows	8 N., 12 A.	"
13932	"	"	"	Dogs	20 N.	"
19924	"	Galveston, Galveston Co.	8/27/42	Dogs	1 ♀	"
20459	"	Hardin Co.	.....	....	14 N.	Southwest Biological Sup. Co.
20465	"	Jefferson Co.	5/19/43	Dog	5 A.	State Bd. Hlth.
14188	"	Kingsville, Kleberg Co.	5/27/38	Cottontail	1 ♂	J. C. Brown
14955	"	"	12/18/38	Deer	1 ♀	"
13709	"	Plum Grove, Liberty Co.	July 1937	....	6 A.	State Bd. Hlth.
13705	"	"	7/2/37	Grass	100 A.	"
13698	"	"	7/3/37	Cows and wooded area	5 A.	"
13692	"	"	7/4/37	Dogs	8 A.	R. M. L.
13702	"	"	7/5/37	Goats	41 A.	St. Bd. Hlth.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
13703	"	"	7/6/37	Bushes	5 A.	"
13693	"	"	7/7/37	Goats	23 A.	R. M. L.
13695	"	"	"	Cows	11 A.	"
13696	"	"	"	Squirrel	5 A.	St. Bd. Hlth.
13701	"	"	"	Cows	7 A.	"
13489	"	"	7/9/37	Dog	5 A.	R. M. L.
13491	"	"	"	Man	4 A.	"
13492	"	"	"	Horse	28 A.	"
13490	"	"	7/10/37	Cows	41 A.	"
13716	"	"	7/12/37	Dog	5 A.	"
13715	"	"	7/14/37	Horse	9 A.	"
13719	"	"	7/14/37	Dog	6 A.	"
13712	"	"	"	Cow	39 A.	St. Bd. Hlth.
13713	"	"	"	Dogs	20 L., 1 N., 2 A.	"
13718	"	"	7/15/37	Goat	22 A.	R. M. L.
13495	"	"	7/16/37	....	3 A.	"
13342	"	"	7/19/37	....	8 A.	"
13826	"	"	7/19/37	Cow	6 A.	"
13827	"	"	"	Dog	15 A.	"
13823	"	"	7/20/37	Goats	18 A.	"
13835	"	"	7/22/37	Dog	8 A.	"
13836	"	"	"	Goat	23 N.	"
13837	"	"	7/23/37	Cow	27 N.	"
13915	Tex.	Plum Grove, Liberty Co.	7/26/37	Cows	21 N., 10 A.	R. M. L.
13916	"	"	"	Dog	19 A.	"
13919	"	"	7/28/37	"	4 A.	"
13920	"	"	"	"	21 A.	"
13921	"	"	"	Cows	27 N.	"
13923	"	"	7/30/37	"	28 A.	"
13926	"	"	"	"	10 A.	"
13929	"	"	8/4/37	"	10 N., 19 A.	"
13933	"	"	"	....	6 N., 11 A.	"
13840	"	"	8/8/37	Cow	6 N., 20 A.	"
13844	"	"	"	Drag	Many N., 1 A.	"
13841	"	Parker Place, Liberty Co.	"	"	200 A.	"
13842	"	"	"	Dog	12 N., 10 A.	"
13843	"	"	"	2 horses	20 N., 15 A.	"
13846	"	Plum Grove, Liberty Co.	8/9/37	Drag	200 N., 1 A.	"
13847	"	"	"	8 cows	2 N., 3 A.	"
13848	"	"	"	Dog	2 N.	"
13849	"	Parker Place, Liberty Co.	"	Drag	450 N., 2 A.	"
13850	"	Howard Ranch, Liberty Co.	"	12 cows	2 N., 54 A.	"
13851	"	Plum Grove, Liberty Co.	8/10/37	Goats	14 N., 2 A.	"
13852	"	"	"	Cows	95 N., 27 A.	"
13853	"	Hughes Kennels, Liberty Co.	"	Dog	27 N., 8 A.	"
13854	"	Ford Place, Liberty Co.	"	"	30 L., 2 N., 3 A.	"
13855	"	Hightower Ranch, Liberty Co.	"	"	21 L., 17 N., 3 A.	"
13857	"	Cleveland, Liberty Co.	"	2 horses	6 N., 4 A.	"
13858	"	"	"	5 dogs	100 N.	"
13859	"	Parker Place, Liberty Co.	8/11/37	Drag	100 N.	"
13861	"	"	"	"	100 N.	"
13862	"	Cleveland, Liberty Co.	"	Horse	5 N., 1 A.	"
13863	"	Parker Place, Liberty Co.	8/12/37	Drag	300 N.	"
13864	"	"	"	2 horses	15 N., 5 A.	"
13866	"	Plum Grove, Liberty Co.	"	Cows	11 N., 3 A.	"
13867	"	"	"	8 dogs	5 N., 5 A.	"
13868	"	Parker Place, Liberty Co.	"	Drag	300 N.	"
13869	"	"	"	Cow	4 N., 5 A.	"
13870	"	"	8/13/37	Drag	675 N.	"
13871	"	"	8/14/37	"	1550 N.	"
13873	"	"	"	Rabbit	Many L., 230 N., 100 A.	"
13872	"	Cleveland, Liberty Co.	"	Dogs	32 N., 7 A.	"
14247	"	"	5/11/38	6 horses	1 N., 68 A.	"
14248	"	"	.....	12 dogs	65 A.	"

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14252	"	"	5/12/38	15 dogs	20 A.	"
14256	"	"	"	Fox squirrel	9 N.	"
14926	"	Dayton, Liberty Co.	9/9/38	13 dogs	8 A.	"
14927	"	"	"	12 cows	1 ♀	"
19954	"	Dayton, Liberty Co.	8/15/42	.....	19 N.	"
19953	"	Liberty Co.	8/16/42	.....	66 N.	"
14264	"	Cleveland, Liberty Co.	5/16/43	10 goats	73 N.	"
13494	"	Montgomery Co.	7/16/37	.....	11 A.	St. Bd. Hlth.
20169	"	Newton Co.	4/4/43	Drag	30 A.	Melvin Kyle
13704	"	San Jacinto Co.	7/4/37	Horses	21 A.	St. Bd. Hlth.
13496	"	"	7/11/37	Cows	12 A.	"
13498	"	"	"	Horses	20 A.	"
13499	"	"	"	Dogs	6 A.	"
13714	"	"	7/14/37	Parker home	6 N., 5 A.	"
13720	"	"	7/15/37	Horse	12 A.	"
13721	"	"	"	Dog	14 A.	"
13722	"	"	"	Flag	19 N., 8 A.	"
13830	Tex.	San Jacinto Co.	7/16/37	Goat	4 A.	St. Bd. Hlth.
13831	"	"	"	Flag	4 A.	"
13832	"	"	"	Horse	15 A.	"
13833	"	"	"	Dog	12 N., 14 A.	"
13829	"	"	7/16-17-30/37	Cows	35 A.	"
13834	"	"	7/21/37	Goats	20 A.	"
13838	"	"	"	.....	5 A.	"
13839	"	"	"	Cow	25 A.	"
13917	"	"	7/23/37	Dogs	6 A.	"
13918	"	"	7/27/37	Cows	16 N.	"
13938	"	"	8/2/37	Cow and dog	9 A.	"
13934	"	"	8/3/37	Dog	16 N., 9 A.	"
14223	"	Manor, Travis Co.	5/1/38	Mule	25 A.	"
14224	"	"	"	Horse	10 A.	"
14225	"	"	"	3 calves	21 N., 9 A.	"

*Amblyomma maculatum* Koch, 1844

(Figs. 5 and 6)

1844. *Amblyomma maculatum* Koch, p. 227.  
 1844. *Amblyomma tigrinum* Koch, p. 227.  
 1844. *Amblyomma rubripes* Koch, p. 227.  
 1844. *Amblyomma ovatum* Koch, p. 228.  
 1844. *Amblyomma triste* Koch, p. 229.  
 1888. *Amblyomma complanatum* Berlese, p. 191.  
 1908. *Amblyomma maculatum* Koch: Banks, p. 39.  
 1911. *Amblyomma maculatum* Koch: Neumann, p. 70.  
 1912. *Amblyomma maculatum* Koch: Hooker, Bishopp and Wood, p. 135.  
 1926. *Amblyomma maculatum* Koch: Robinson, p. 40.  
 1936. *Amblyomma maculatum* Koch: Oudemans, p. 504.

## Female

*Body*: Length, unengorged, from 3.70 to 4.40, width from 2.40 to 2.85. Engorged examples may become as long as 18.00 and as wide as 13.00. Oval, a little narrower in front. Scutum less than half the body length. Marginal groove complete and continuous across all the festoons. Festoons often with a small, terminal nub visible from above.

*Scutum*: Length 1.8 to 2.20, width 1.60 to 2.00. Sub-triangular, narrowed posteriorly, rounded terminally; antero-lateral border very convex. Scapulae long, pointed. Eyes a little convex. Cervical grooves moderately deep anteriorly, shallow, and disappearing posteriorly. Ornate, with the light and dark color pattern contrasting sharply. Punctations numerous, large and small ones intermingled, the former more numerous in anterior areas.

*Capitulum*: Length from 1.00 to 1.25, width of basis 0.73. Basis sub-rectangular in shape, postero-lateral corners a little protruding, salient. Porose areas large, convex, a little divergent anteriorly. Surface smooth, shining, punctate. Palpi long, wider at article 3; hairs few and short.

*Hypostome*: Long, mildly notched apically. Dentition 3/3, though examination of numerous

specimens shows a tendency for a 4/4 pattern. One female examined was definitely 4/4 but the teeth of the fourth row (median) were small. Length 0.78.

*Legs:* Long and large, I longer but smaller than the others. Apical spurs absent on tarsus I, present on II, III and IV. Metatarsi II, III and IV with long, paired terminal spurs, directed distad. Length of tarsus I, 1.11; metatarsus, 0.99. Length of tarsus IV, 0.78; metatarsus, 1.20.

*Coxae:* Coxa I with a very short internal and a long, pointed external spur. Coxa II with a broad, short, flat spur; III and IV with similar spurs, but progressively smaller. All coxae with a few long, fine hairs.

*Spiracular plate:* Large, concave, with the frame well sclerotized. Length 0.87, width 0.66.

*Genital aperture:* Situated at the level of the intervals between coxae II and III.

#### Male

*Body:* Length from 3.40 to 5.00, width from 2.40 to 3.00. A medium-sized tick, oval, wider behind.

*Scutum:* Often with the surface mildly concave between the eyes. Lateral grooves deep, complete, starting near the eyes and with their continuation limiting the long, well-defined festoons. Ventral scutes extending over the margin and visible from above. Cervical grooves deep in front, shallow behind and disappearing back of the eyes. Scapulae long with the interval between them deep. Punctations numerous and deep in the area within the marginal groove, sparse on the festoons. Eyes a little convex. Ornate with numerous, mostly connected lineal spots of golden white (see the figure).

*Capitulum:* Length 0.80 to 1.20, width of basis about 0.63. Basis sub-rectangular, longer than in many species; dorsal surface convex, a little irregular, punctate; postero-lateral corners a little protruding, salient, forming short cornua. Palpi long, widest on article 3. Hairs moderate in number, short and fine. Length 0.66.

*Hypostome:* Spatulate, long, notched terminally. Dentition 3/3; rarely there is a fourth pair more or less complete. Length from 0.66 to 0.78.

*Legs:* Long and large. Leg I smaller. Apical spurs present on II, III and IV, absent on I. Long, paired, terminal spurs present on metatarsi II, III, IV, absent on I. Hairs long and fine. Length of tarsus I, 0.84; metatarsus, 0.84. Length of tarsus IV, 0.60; metatarsus, 1.02.

*Coxae:* Coxa I with long, pointed external spur; internal spur short, almost negligible. Coxae II and III with short, broad, flat spurs. Coxa IV with a long, pointed internal spur. All coxae with a few long, fine hairs.

*Spiracular plate:* Large, a little convex, with the frame well sclerotized. Length 0.69, width 0.51.

*Genital aperture:* Situated between coxae II.

#### Nymph

*Body:* Unengorged body pyriform, narrow; with the scutum reaching about one-third the length. Length 1.44, width 0.93.

*Scutum:* Length 0.57, width 0.63. Slightly wider than long, broadly rounded behind; widest back of the middle. Scapulae rounded and covered by the sides of the basis capituli. Cervical grooves moderately deep anteriorly, then continued as shallow valleys to near the postero-lateral margins. Eyes pale, nearly flat. Surface faintly shagreened. Punctations sparse. Hairs absent.

*Capitulum:* Length 0.36, width of basis 0.315. Basis triangular, with the lateral points protruding outside of the scapulae. Ventral surface of basis with two distinct retrograde spurs. Posterior margin convex. Surface nearly flat (dorsally), smooth, shining, impunctate. Palpus long, narrow at the base, broadest at about the suture between 2 and 3. Combined length of 2 and 3, 0.20. Hairs few and moderately long.

*Hypostome:* Spatulate, rounded terminally. Dentition 2/2. Length about 0.17.

*Legs:* Relatively a little shorter and smaller than in the adults. All tarsi with apical ventral spurs absent. Paired terminal spurs absent on all metatarsi. Length of tarsus I, 0.35; metatarsus, 0.18. Length of tarsus IV, 0.24; metatarsus, 0.21.

*Coxae:* Coxa I with only moderate internal spur. Coxa II with one very short, broad, flat spur. Coxae III and IV without spurs. All coxae smooth and shining and with a few fine hairs.

*Spiracular plate:* Large, flat, and with frame little sclerotized. Length 0.23, width 0.15.

#### Larva

*Capitulum:* Length 0.17, width of basis 0.16. Basis sub-triangular, wide, with the lateral points extending outside over the scapulae; posterior margin rounded. Surface smooth, shining, faintly shagreened, impunctate. Palpi short, smooth, and with a few fine hairs. Length 0.086.

*Hypostome:* Spatulate, rounded terminally. Dentition 2/2. Length about 0.11.



*Scutum*: Length 0.24, width 0.36. Very broadly rounded behind. Widest a little back of the middle. Cervical grooves shallow, nearly parallel. Surface smooth, shining, shagreened, impunctate.

*Coxae*: Spurs faintly visible. Each coxa with a single short, triangular spur, that on I largest, those on II and III progressively smaller. Hairs absent.

#### DISTRIBUTION AND HOSTS

The type was from "Carolina"; the type host is unknown.

In the United States the species is apparently established only in the areas bordering the Gulf of Mexico, and along the Atlantic Coast. Bishopp and Hixson (1936) state that this tick is seldom found in numbers more than 100 miles inland and that its distribution suggests "that rather high rainfall, humidity and temperature are necessary to its existence." Early records of *A. maculatum* from outside the present known normal limits of distribution are as follows: from Virginia (Niles, 1898, as *Dermacentor occidentalis*); from Memphis, Tenn. (Hunter and Hooker, 1907); from Tulare County, Calif. (Banks, 1908); from Dallas, Texas (Hooker et al, 1912). We have two recent records in the same category, i.e., a female from unknown host, Willcox, Arizona, Sept. 22, 1942 (L. P. Wehrle) and an engorged female from a dog, Weathers, Okla., Sept. 11, 1942 (Glen M. Kohls). Numerous records kindly supplied by Dr. Paul L. Piercy, Animal Parasitologist, Texas Agricultural Experiment Station, are entirely in keeping with those of this Laboratory and the map of Hooker et al (1912, p. 143). Neumann (1899, p. 252) records *A. maculatum* from Peru and Paraguay, and Neumann again (1911, p. 70) from Mexico, Ecuador, Chile and Patagonia. Robinson (1926, p. 44) records it from Brazil and Uruguay. Lahille (1905, pp. 152-153) reports that the species is common in Argentina. Newstead (1909, p. 445) reports males and females from Jamaica. Robinson (1926, p. 44) adds Colombia because of specimens donated by Dr. H. Gadow, and the Rocky Mountain Laboratory has several lots from Colombia sent by Dr. Luis Patiño-Camargo.

The adults attack a variety of the larger animals, both wild and domestic. Man is attacked occasionally. Larvae and nymphs attack birds principally but are found also on the smaller wild mammals. Hooker et al (1912) list as hosts of the adult ticks, cattle, sheep, horse, goat, dog, wolf and man, and of nymphs, meadow lark, red-winged blackbird, fox and jackrabbit. Bishopp (1912) listed collections of nymphs in southern Texas from meadow lark, *Sturnella magna*; red-winged blackbird, *Agelaius phoeniceus*; Brewer's blackbird, *Euphagus cyanocephalus*; and jack rabbit, *Lepus californicus merriami*. Hixson (Bishopp and Hixson, 1936, and Hixson, 1940), reporting from the vicinity of Valdosta, Ga., found the meadow lark to be the most important host of the immature forms during the fall and winter months. Larval hosts listed were meadow lark, *Sturnella magna*; bobwhite, *Colinus virginianus*; southern fox squirrel, *Sciurus niger niger*; eastern cotton rat, *Sigmodon hispidus hispidus*; towhee, *Pipilo erythrophthalmus*; field sparrow, *Spizella pusilla pusilla*; southern gray squirrel, *Sciurus carolinensis carolinensis*; house wren, *Troglodytes aedon*; brown thrasher, *Toxostoma rufum*; kid; lamb; mocking bird, *Mimus polyglottos*; blue jay, *Cyanocitta cristata*; loggerhead shrike, *Lanius ludovicianus ludovicianus*; and roof rat, *Rattus rattus alexandrinus*. Listed as host of the nymph were meadow lark, bobwhite, eastern cotton rat, southern fox squirrel, sheep, calf, towhee, house wren, American robin, *Turdus migratorius*; and the white-throated sparrow, *Zonotrichia albicollis*. Meadow larks and bobwhites were regarded as the most im-

portant hosts. Peters (1936) recorded the Carolina wren, *Thryothorus ludovicianus*, and the Red-eyed Towhee, *Pipilo erythrophthalmus*, as hosts in South Carolina without mention of stage or stages found.

For hosts outside of the United States see Robinson (1926).

Following are the records for the United States of the Rocky Mountain Laboratory:

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
16004	Ala.	Auburn, Lee Co.	7/26/39	Cow	3 ♀	Dr. E. W. Price
19377	Fla.	Bombay, Holmes Co.	9/6/41	Ears of cattle	2 ♀	L. E. Swanson
17153	Ga.	Nashville, Berrien Co.	7/29/40	Sheep	192 A.	Mr. Van Wheless
17040	"	"	8/9/40	"	75 A.	"
19650	"	Savannah, Chatham Co.	.....	.....	5 A.	R. M. L.
16008	"	Albany, Dougherty Co.	8/14/39	Dogs	6 A.	Geo. G. Brigham
16658	"	Ludowici, Long Co.	7/12/39	Sheep	4 A.	Dr. E. W. Price
14172	"	"	.....	.....	100 A.	B. A. I.
20495	Miss.	Keester Field, Harrison Co.	8/29/43	Man	1 ♂	F. N. Young, Jr.
14171	"	Jackson, Hinds Co.	.....	.....	50 A.	B. A. I.
14078	"	"	.....	Cattle?	Many	Dr. E. W. Price
19933	Okla.	Weathers, Pittsburg Co.	9/11/42	6 dogs	1 ♀	R. M. L.
13935	Tex.	Tarleton, Prairie, Erath Co.	8/3/37	Cow	3 A.	"
19924	"	Galveston, Galveston Co.	8/27/42	Dogs	7 A.	Kohls and McGregor
20459	"	Saratoga, Hardin Co.	.....	.....	1 ♂	Southwest Biological Sup. Co.
17235	"	Beaumont, Jefferson Co.	9/3/40	Mule	1 A.	R. M. L.
17239	"	"	9/5/40	Horses, mules, cattle	200 A.	"
17241	"	"	9/6/40	Horses and cattle	85 A.	"
11437	Tex.	Kingsville, Kleberg Co.	10/17/35	Horse	7 A.	R. M. L.
11144	"	Riviera, Kleberg Co.	10/19/35	Dog	6 A.	"
11443	"	"	"	Horse	2 A.	"
14832	"	Kingsville, Kleberg Co.	7/18/38	Coyote	1 A.	J. C. Brown
13826	"	Liberty Co.	7/19/37	Cow	1 A.	St. Bd. Hlth.
13917	"	Cleveland, Liberty Co.	7/23/37	Dogs	2 A.	"
13930	"	"	8/4/37	Dog	2 ♀	R. M. L.
13850	"	"	8/9/37	12 cows	23 A.	"
13857	"	"	8/10/37	2 horses	1 ♀	"
13866	"	"	8/12/37	Cows	1 A.	"
14926	"	Dayton, Liberty Co.	8/9/38	13 dogs	23 A.	"
14927	"	"	"	12 cows	86 A.	"
14928	"	"	"	Horse	7 A.	"
14930	"	Cleveland, Liberty Co.	8/12/38	20 horses	40 A.	"
14929	"	Dayton, Liberty Co.	"	Drag	46 A.	"
14931	"	"	8/13/38	Drag	60 A.	"
14932	"	Cleveland, Liberty Co.	"	45 horses	62 A.	"
17228	"	Dayton, Liberty Co.	8/31/40	Drag	62 A.	"
17231	"	Dayton, Liberty Co.	9/2/40	Cattle and dogs	18 A.	R. M. L.
17234	"	"	"	Drag	48 A.	"
19891	"	"	7/24/42	Cattle	100 A.	T. McGregor
19954	"	"	8/15/42	.....	192 A.	"
19953	"	Liberty, Liberty Co.	8/16/42	.....	214 A.	"
17242	"	Inez, Victoria Co.	9/9/40	Cattle	75 A.	R. M. L.
17245	"	Victoria, Victoria Co.	"	"	85 A.	R. M. L.
17247	"	"	"	"	66 A.	"

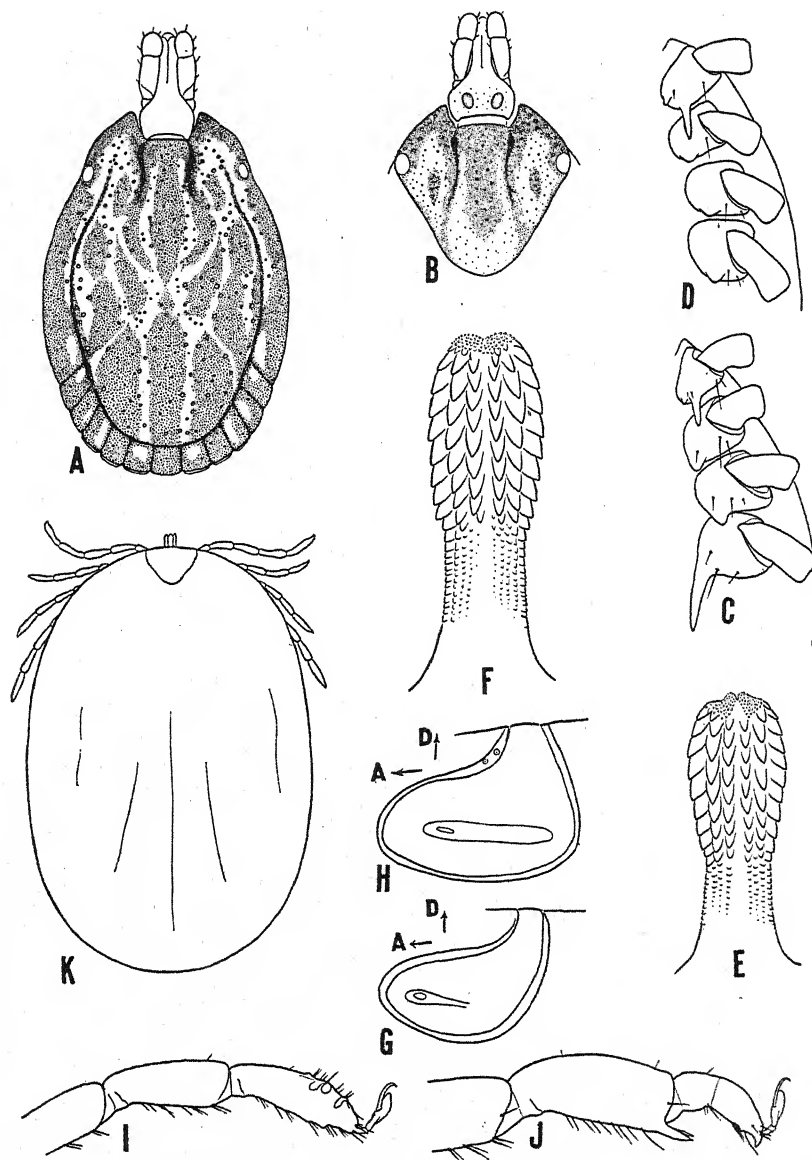


FIG. 5. *Amblyomma maculatum* Koch. A. Capitulum and scutum, male. B. Capitulum and scutum, female. C. Coxae of male. D. Coxae of female. E. Hypostome of male. F. Hypostome of female. G. Spiracular plate of male. H. Spiracular plate of female. I. Leg I of male. J. Leg IV of male. K. Engorged female.

*Amblyomma dissimile* Koch 1844

(Figs. 7 and 8)

- 1844. *Amblyomma dissimile* Koch, p. 225.
- 1844. *Amblyomma irroratum* Koch, p. 225.
- 1844. *Amblyomma adpersum* Koch, p. 226.
- 1844. *Amblyomma infumatum* Koch, p. 228.
- 1844. *Ixodes flavidus* Koch, p. 233.
- 1844. *Ixodes humanus* Koch, p. 233.

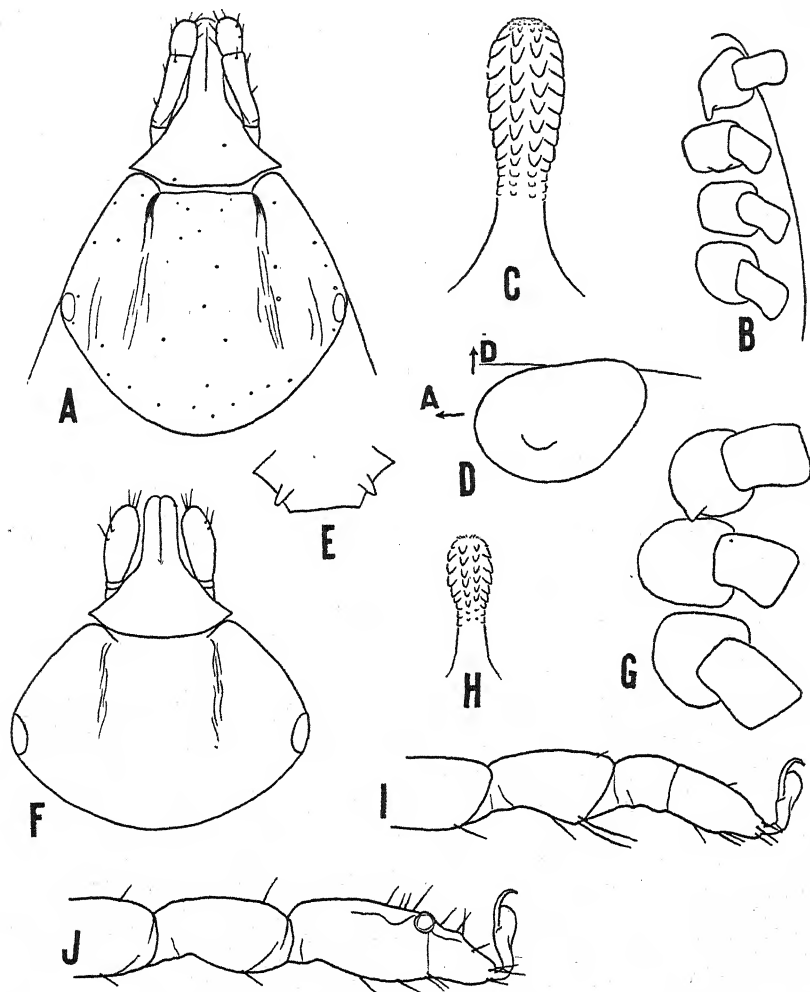


FIG. 6. *Amblyomma maculatum* Koch. A. Capitulum and scutum of nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Spurs on venter of nymphal basis capituli. F. Capitulum and scutum of larva. G. Coxae of larva. H. Hypostome of larva. I. Leg IV of nymph. J. Leg I of larva.

1846. *Ixodes pulchellus* Lucas, p. 61.

1886-1893. *Ixodes boarum* Stoll, p. 18.

1911. *Amblyomma dissimile* Koch: Neumann, p. 72.

1912. *Amblyomma dissimile* Koch: Hooker, Bishopp and Wood, p. 130.

1926. *Amblyomma dissimile* Koch: Robinson, p. 163.

1936. *Amblyomma dissimile* Koch: Oudemans, p. 471.

#### Female

**Body:** Unengorged body about 3.6 long by 3.0 wide; moderately well sclerotized. Much engorged body, 15.00 long by 9.00 wide; wider posteriorly.

**Scutum:** Length from 1.74 to 2.28; width from 2.10 to 2.58. Robinson (1926) gives size as  $2.45 \times 2.85$ . Sub-triangular, a little wider than long, postero-lateral sides nearly straight. Cervical grooves sigmoid-shaped, deep anteriorly, fading out behind. Punctations few, large and small ones intermingled. Ornate, with irregular spots in antero-lateral areas, and a large, pale spot in the median posterior area. Eyes large, flat, pale.

**Capitulum:** Length 1.14 to 1.41, width of basis 0.75 to 0.87. Robinson (1926) gives length as 1.6. Sub-rectangular, lateral margins convex. Surface sparingly punctate. Porose areas large,



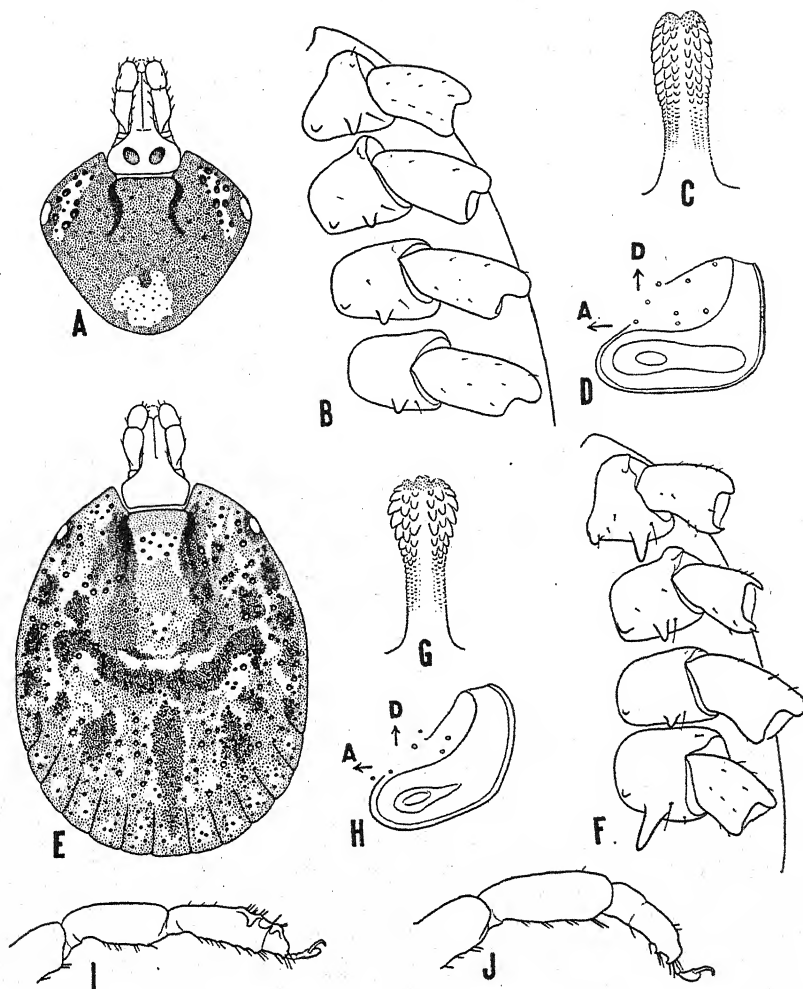


FIG. 7. *Amblyomma dissimile* Koch. A. Scutum and capitulum of female. B. Coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Coxae of male. G. Hypostome of male. H. Spiracular plate of male. I. Leg I of male. J. Leg IV of male.

oval, divergent anteriorly, and separated by about the shorter axis of one. Palpi long, clavate, laterally compressed; combined length of 2 and 3, 0.78 to 0.96.

*Hypostome*: Long, spatulate, notched apically. Dentition 3/3. Length about 0.69.

*Legs*: Of medium length, slender, smooth. Apical ventral spur faint on tarsus I, distinct on II, III and IV. Sub-apical ventral spur absent on I; small on II, III and IV. Length of tarsus I, 0.93; metatarsus, 0.72. Length of tarsus IV, 0.75; metatarsus, 0.75.

*Coxae*: In unfed specimens, coxae situated distant from the lateral margins of the body. A short, external spur present on all coxae. Coxae I, II and III with very short internal spurs; absent on IV. Robinson (1926) describes and figures the internal spur as present on IV.

*Spiracular plate*: Large, heavily chitinized, surface depressed. Greatest length 0.78, greatest width 0.60.

*Genital aperture*: Situated between coxae II.

#### Male

*Body*: Length from 2.75 to 4.50, width from 2.35 to 3.75. Broad oval, a little wider behind. Disparity in sizes is notable and small specimens have the color pattern less distinct.

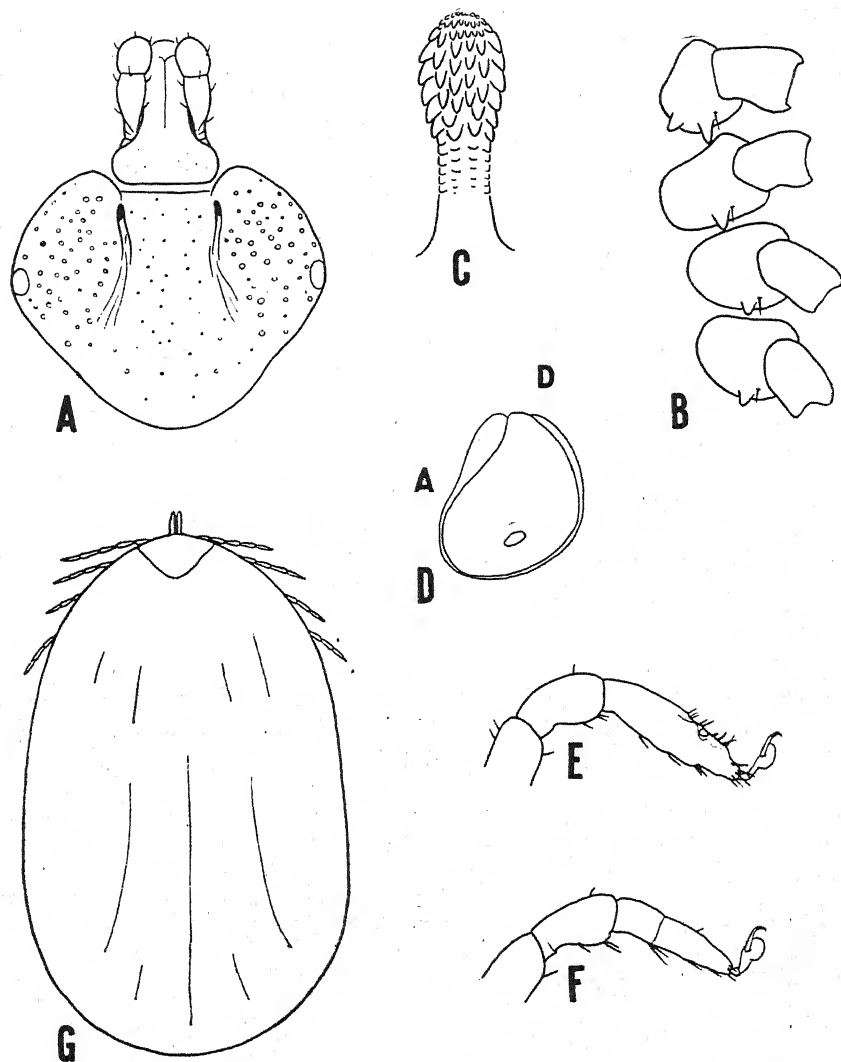


FIG. 8. *Amblyomma dissimile* Koch. A. Capitulum and scutum of nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Leg I of nymph. F. Leg IV of nymph. G. Engorged female.

**Scutum:** Smooth, mildly convex, declivitous at sides and posteriorly. Lateral grooves absent. Cervical grooves deep and short. Ornate, with reddish-brown stripes on a pale yellow pattern tinged with rose-red. Pattern shown in the figure. Punctations large and small, intermingled, numerous in both light and dark spots. Festoons long.

**Capitulum:** Length from 0.84 to 1.32; width of basis from 0.60 to 0.87. Essentially as in the female. Combined length of palpal articles 2 and 3 from 0.57 to 0.84.

**Hypostome:** Long, spatulate, faintly notched and sinuous apically. Dentition 3/3. Length about 0.63.

**Legs:** Essentially as in the female. Length of tarsus I, 0.84; metatarsus, 0.72. Length of tarsus IV, 0.75; metatarsus, 0.9.

**Coxae:** All coxae with two spurs, with external spurs larger. External spurs I and IV about equal, II and III equal but smaller. Internal spurs progressively smaller from I to IV, that on IV very small.

**Spiracular plate:** Large and heavily sclerotized. Greatest length 0.84; greatest width 0.45.

**Genital aperture:** Situated between coxae II.

## Nymph

Well engorged specimens are 3.9 long and 2.94 wide. No unfed specimens are available.

*Capitulum*: Length 0.42, width of basis 0.3. Basis convex on top with the lateral profile margins curved. Posterior margin a little convex; postero-lateral corners faint. Palpi moderate in length; surface irregular. Length 0.3.

*Scutum*: Length 0.66, width 0.96. Much wider than long. Cervical grooves sigmoid, about half the length of the scutum; deep anteriorly. Surface faintly irregular. Punctations moderate in number, larger and deeper in the antero-lateral areas.

*Hypostome*: Spatulate, rounded apically. Dentition 3/3. Length about 0.87.

*Legs*: Ventral spurs on tarsi absent. Length of tarsus I, 0.39; metatarsus, 0.21. Length of tarsus IV, 0.33; metatarsus, 0.24.

*Coxae*: Coxa I with two small spurs; II, III and IV with one small spur.

*Spiracular plate*: Much less sclerotized than in the adults. Greatest length 0.24; width, 0.18.

## DISTRIBUTION AND HOSTS

The only United States records of the species are those of Bequaert (1932) from southern Florida, i.e., from gopher snake, *Spilotes corais couperi* (Holbrook), Sebastian, Indian River Co., February, 1909, and from pigmy or ground rattler, *Sistrurus miliaris* (Linnaeus), Boca Ratone, Palm Beach Co. (1931).

The species has an extended southern range which, according to Bequaert, includes Mexico, Guatemala, British Honduras, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Trinidad, Grenada, Tobago, British Guiana, Peru and Brazil.

Known hosts include 20 different kinds of Reptilia and Amphibia. In addition to these, Robinson (1926) includes a record from cow and from sheep, and Neumann (1911) mentions the capybara, "*Hydrochoerus hydrochoeris* (L.) (*H. capybara* Erxl.)."

*Amblyomma tuberculatum* Marx, 1894

(Figs. 9 and 10)

1894. *Amblyomma tuberculatum* Marx, p. 314.

1908. *Amblyomma tuberculatum* Marx: Banks, p. 38.

1911. *Amblyomma tuberculatum* Marx: Neumann, p. 74.

1912. *Amblyomma tuberculatum* Marx: Hooker, Bishopp and Wood, p. 123.

1926. *Amblyomma tuberculatum* Marx: Robinson, p. 174.

1932. *Amblyomma tuberculatum* Marx: Bequaert, p. 778.

## Female

*Body*: The largest known species of the genus in the United States. Length 7.0 to 7.5, width 5.5 to 6.00. Broad oval, heavily sclerotized. Scutum reaching about two-fifths the length of body.

*Scutum*: Length 3.6, width 4.2. Shape broadly cordiform. Cervical grooves deep and curved. Ornate with dark-brown markings on a yellow-white ground. Pattern shown in the figure. Fine punctations very numerous and with a few large ones in the antero-lateral areas. Eyes small, flat, pale.

*Capitulum*: Length 2.3 to 2.60, width of basis 1.56. Basis sub-rectangular, posterior margin straight. Porose areas small, oval, divergent anteriorly, widely separated. Palpi long, relatively longer than those in the male. Combined length of 2 and 3, 1.59.

*Hypostome*: Spatulate, long, with the denticles 4/4, occupying two-fifths the total length. Length about 1.08.

*Legs*: Apical ventral spurs present on all the tarsi. Sub-apical ventral spurs present on tarsi II, III and IV. Length of tarsus I, 1.14; metatarsus, 1.10. Length of tarsus IV, 1.20; metatarsus, 1.53.

*Coxae*: Coxae I to IV each with two short, broad, flat spurs; internal spur on IV smaller.

*Spiracular plate*: Large, heavily chitinized, with the surface much depressed.

*Genital aperture*: Opposite the posterior borders of coxae II.

## Male

*Body*: Length 6.2, width 5.5. Broad oval, wider behind, sides evenly rounded.

*Scutum*: Smooth, convex, all margins declivitous. Cervical grooves long, curved, deep in their anterior ends. Marginal grooves absent. Ornate, with dark-brown spots, mainly separated, on a dull, yellow-white ground. Pattern shown in the figure. Punctations very numerous and fine, and with a few large ones intermingled. Festoons short; ventral scutes protruding farther than the festoons, leaving narrow shelves visible from above.

*Capitulum*: Length 1.8, width of basis 1.23. Essentially as in the female but with the palpi relatively shorter. Combined length of palpal articles 2 and 3, 1.05.

*Hypostome*: Spatulate, faintly notched apically. Denticles 4/4. Length about 1.20.

*Legs*: Apical ventral and sub-apical spurs present on all tarsi. Length of tarsus I, 0.93; metatarsus, 0.93. Length of tarsus IV, 0.84; metatarsus, 1.50.

*Coxae*: Coxae I to IV each with two short, broad, flat spurs. Internal spur on IV smaller and situated on the median edge of the coxa.

*Genital aperture*: Situated between coxae II.

#### Nymph

*Capitulum*: Length 0.93, width of basis 0.66. Basis sub-quadrate, convex, with sides not margined but merging into the lateral walls. Posterior margin nearly straight. Palpi long, widest on article 3.

*Scutum*: Length 1.44, width 1.74. Broadly cordiform with the postero-lateral margins a little flattened. Cervical grooves curved and deep. Ornate, with dark brown markings on dark yellowish-white ground. Pattern shown in the figure. Surface very finely pebbled. Large, deep punctations numerous in the lateral areas, small punctations in the median areas.

*Hypostome*: Shape as in the adults. Dentition 3/3. Length about 0.36.

*Legs*: All tarsi with the apical, ventral spur present as in adults, but small. Length of tarsus I, 0.60; metatarsus, 0.45. Length of tarsus IV, 0.60; metatarsus, 0.63.

*Coxae*: Coxae I and II each with two broad, short, flat spurs; internal spurs smaller. Coxae III and IV each with one external spur; internal spurs lacking.

*Spiracular plate*: Large and heavily sclerotized. Surface much depressed. Length and width about equal—0.33.

#### Larva

From cast skins of fully fed larvae we are able to describe the larva in part.

*Scutum*: Length 0.45, width 0.57. Cervical grooves long, extending about three-fourths the length of the scutum, deeper anteriorly. Surface impunctate.

*Capitulum*: Sub-triangular with rounded points at the sides. Length 0.312, width of basis 0.221. Combined length of articles 2 and 3, 0.18.

*Hypostome*: Dentition 2/2. Length 0.192.

*Coxae*: Coxa I with two small spurs; II and III each with one small spur.

#### DISTRIBUTION AND HOSTS

This species is known in this country only from Alabama, Georgia and Florida. Bequaert (1932) stated:

*A. tuberculatum* is, in the adult and nymphal stages, a common and specific parasite of the gopher turtle, *Gopherus polyphemus* (Daudin). Most probably its range coincides with that of the host, which occurs throughout the coastal plain of the Southeastern United States. L. Stejneger and T. Barbour (1923, A Check List of North American Amphibians and Reptiles, 2d ed., p. 137) give the distribution of this gopher turtle as "Coast from southern South Carolina to Florida and the Mississippi River north into southern Arkansas." Dr. Barbour tells me that it does not occur in the extreme southern part of Florida and that it certainly was never indigenous in Cuba. Neumann (1899) recorded a male of *A. tuberculatum*, at the Paris Museum, collected by Gundlach in Cuba; but this specimen must have been taken off a gopher turtle brought from the mainland and kept in captivity.

Previous, definite locality records of *A. tuberculatum* are as follows: Crescent City, Putnam Co., Florida (type locality; Marx and Hubbard, 1894). Enterprise, Volusia Co., Florida (Banks, 1904). Hawthorn, Alachua Co., Florida (Hooker, Bishopp and Wood, 1912). Southern Alabama (Hooker, 1909; Robinson, 1926).

I have seen five different lots of this tick, all taken off *Gopherus polyphemus*, from the following localities: Lakeland, Polk Co., Florida (Am. M. N. H. and W. T. Davis Coll.). La Grange, Brevard Co., Florida (W. T. Davis Coll.). Mt. Pleasant, Gadsden Co., Florida (W. T. Davis Coll.). South Carolina, without more definite locality (off a turtle brought to the Bronx Zoölogical Park.—W. T. Davis Coll.).



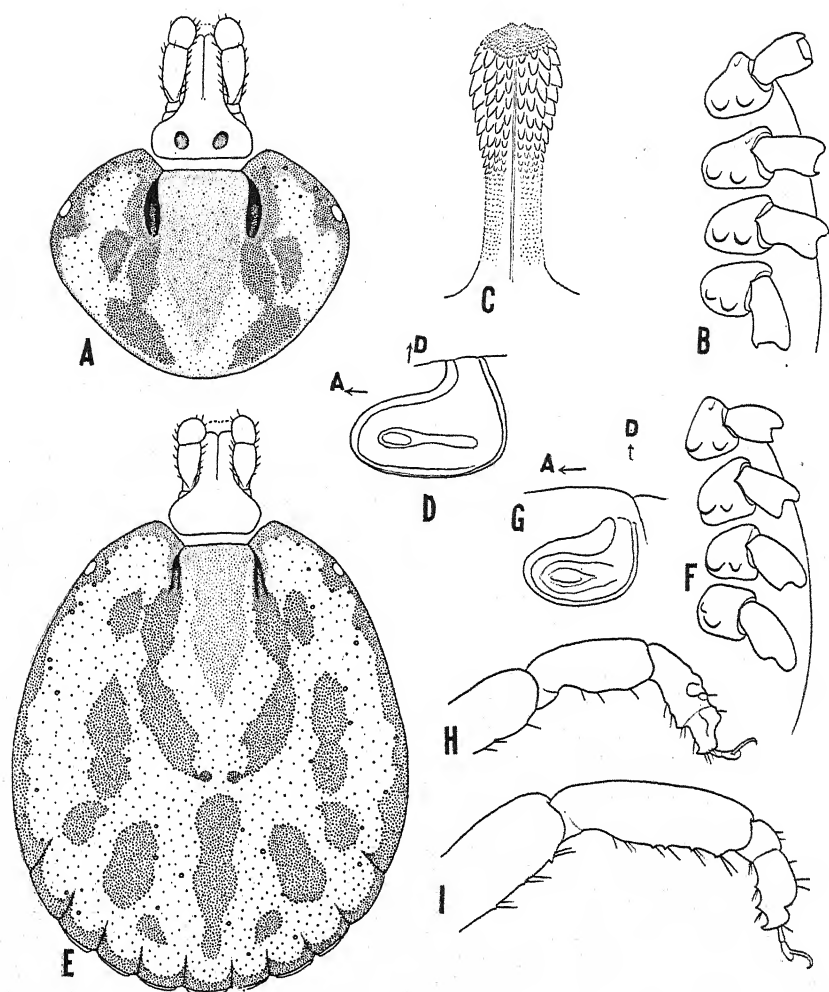


FIG. 9. *Amblyomma tuberculatum* Marx. A. Capitulum and scutum of female. B. Coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Coxae of male. G. Spiracular plate of male. H. Leg I of male. I. Leg IV of female.

The southern meadowlark, *Sturnella magna argutula*, has been recorded as a host in Florida, without mention of the stage of the tick.

Hooker, Bishopp and Wood (1912) stated, "engorged larvae have been collected in large numbers from dogs and rabbits and in smaller numbers from cattle and two birds of prey, namely, the owl and the hawk."

Young and Goff (1939) give the following records, all from *Gopherus polyphemus* in Florida: Miami, May 20, 1933; Leesburg, May 4, 1938, and Gainesville, November 28, 1936.

The Rocky Mountain Laboratory collection contains specimens as follows: 18545, *Gopherus polyphemus*, Florida, April 23, 1939, 4 ♂ (Amer. Mus. Nat. History); 19833, *Sceloporus undulatus undulatus*, Silver Springs, Marion Co., Florida, February 28, 1942, 5 nymphs (J. Frenkel); 16266, *Geomys floridanus*, Newman's Lake, Alachua Co., Florida, December 9, 1939, several larvae (B. A. Barrington) and

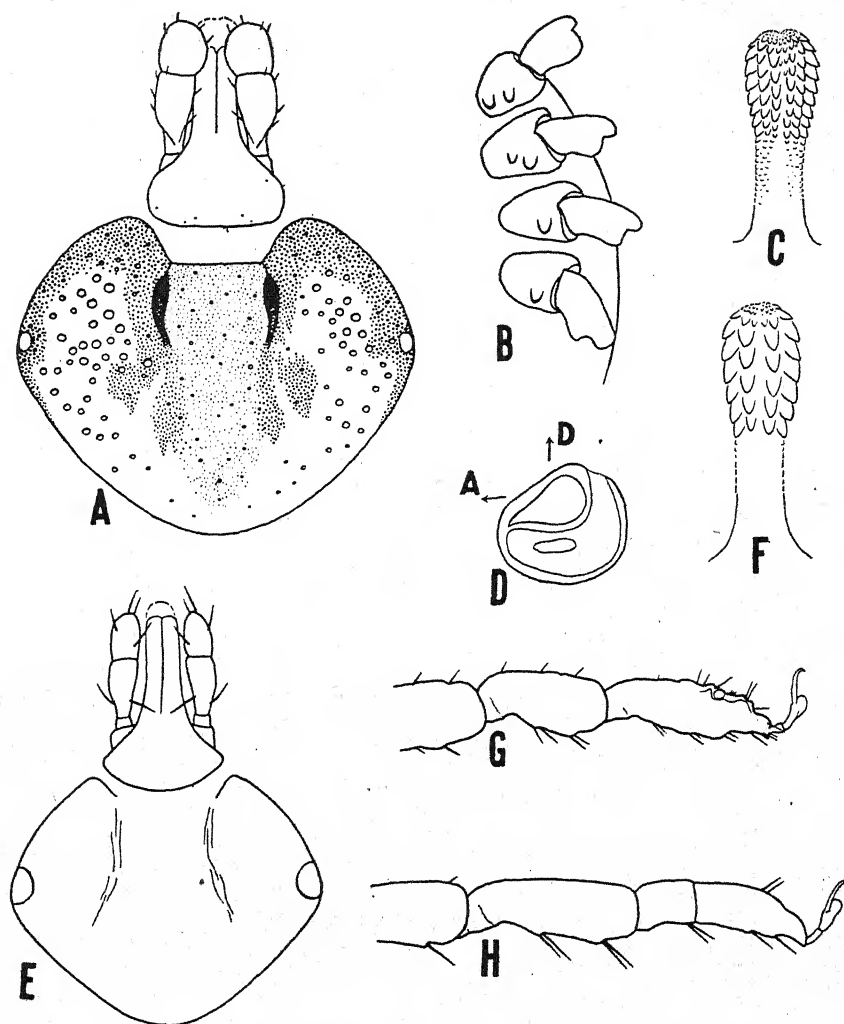


FIG. 10. *Amblyomma tuberculatum* Marx. A. Capitulum and scutum of ornate nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Capitulum and scutum of larva. F. Hypostome of larva. G. Leg I of nymph. H. Leg IV of nymph.

19651, Broxton, Coffee Co., Georgia, host and date unknown, 1 ♂, 1 ♀ (Dr. G. Brigham).

*Amblyomma inornatum* (Banks, 1909)

(Fig. 11)

1909. *Aponomma inornata* Banks, p. 171.

1939. *Amblyomma philipi* Cooley and Kohls, p. 44.

Female

**Body:** Banks (1909) in describing the species gives the length as 8 mm. We have neither unengorged nor fully engorged specimens. One slightly fed specimen is oval, narrower in front.

**Scutum:** Length 1.36 to 1.62, width 1.44 to 1.56. Inornate. About as long as wide, widest a little in front of the middle. Broadly rounded behind, with the postero-lateral margins a little convex. Cervical grooves deep in front, then continuing as valleys and disappearing without reaching the postero-lateral margins. Punctations numerous, moderate in size. Some specimens have smooth elevations without punctations near the eyes which suggest lateral carinae. Eyes large, pale, slightly convex and margined by small punctations.

*Capitulum*: Length 0.90 to 1.02, width of basis 0.57 to 0.66. Basis sub-triangular, with the posterior margin straight, postero-lateral edges salient. Porose areas large, oval, divergent anteriorly and well separated. Surface smooth, shining, punctate. Palpi long, narrow proximally. Postero-dorsal ridge moderate. Hairs moderate in number.

*Hypostome*: Long, spatulate, faintly notched apically. Dentition 3/3. Length about 0.54.

*Legs*: Moderate in length and size. Apical ventral spur absent on tarsus I, present on II, III and IV. Sub-apical ventral spurs absent. Length of tarsus I, 0.54; metatarsus, 0.36. Length of tarsus IV, 0.48; metatarsus, 0.42.

*Coxae*: Coxa I with a short internal and a moderately long external spur. Coxae II, III and IV each with one broad, triangular, external spur. Faint trochantal spurs present on legs I and II. All coxae with a few hairs.

*Spiracular plate*: Moderate in size, flat, well sclerotized. Length 0.45, width 0.36.

*Genital aperture*: Situated between coxae II.

#### Male

*Body*: Length 1.86 to 2.04, width 1.32 to 1.44. Shape almost pyriform.

*Scutum*: Inornate. Smooth and shining. Lateral grooves distinct, starting back of the eyes and limiting the long festoons. Cervical grooves deep and short. Eyes small, pale and only slightly convex. Posterior median surface in front of the festoons more elevated than the festoons. Punctations numerous, large and present also on the festoons. Hairs absent.

*Capitulum*: Length 0.54 to 0.60, width of basis 0.37 to 0.39. Basis sub-triangular, cornua a little pointed, posterior margin between the cornua nearly straight, salient. Surface smooth, shining, punctate. Hairs absent. Palpi moderate in length, much broader distally, smooth, shining, impunctate, and with a few short hairs.

*Hypostome*: Spatulate, broad distally, very faintly notched apically. Dentition 3/3. Length about 0.3.

*Legs*: Terminal ventral spur absent on tarsus I, present on II, III and IV. Sub-apical ventral spurs absent. Length of tarsus I, 0.48; metatarsus, 0.30. Length of tarsus IV, 0.42; metatarsus, 0.375.

*Coxae*: All coxae as in the female. Faint trochantal spurs present on I and II in some specimens.

*Spiracular plate*: Large, flat, moderately sclerotized. Length 0.48, width 0.195.

*Genital aperture*: Situated between coxae II.

*A. inornatum* rather closely resembles *A. parvum* Aragão, 1908. *A. inornatum* is smaller, lacks the retrograde spur on the venter of the male palpal article 1, which in the female is poorly developed, and has the much larger punctations on the scuta of both sexes. In the males the most notable differences are in the palpi in dorsal view. In *parvum* the palpus is short and broad, and on article 2 has the postero-dorsal ridge prominent and sometimes overhanging.

#### HOSTS AND DISTRIBUTION

*Amblyomma inornatum* was described from dog at Corpus Christi, Texas, and from the rabbit, Victoria, Texas. No type specimens were so labelled but Dr. Bishopp has shown the senior author specimens believed to be the ones which Banks had before him when the species was described. The above descriptions are based on specimens in the collections of the Rocky Mountain Laboratory taken at Kingsville, Texas.

All other known records are tabulated below.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14069	Texas	Kingsville	6-10-38	Rabbit	1 ♀	J. C. Brown
14190	"	"	3-24-38	Rabbit	1 ♂	"
14295	"	"	5-24-38	Jackrabbit	1 ♂	R. M. L.
14329	"	"	5-25-38	<i>Canis</i> sp. (coyote)	4 ♂, 1 ♀	R. M. L.
14330	"	"				
14331	"	"				
14332	"	"				
17247	"	27 mi. N.E. of Victoria	9-9-40	Cow	2 ♀	"

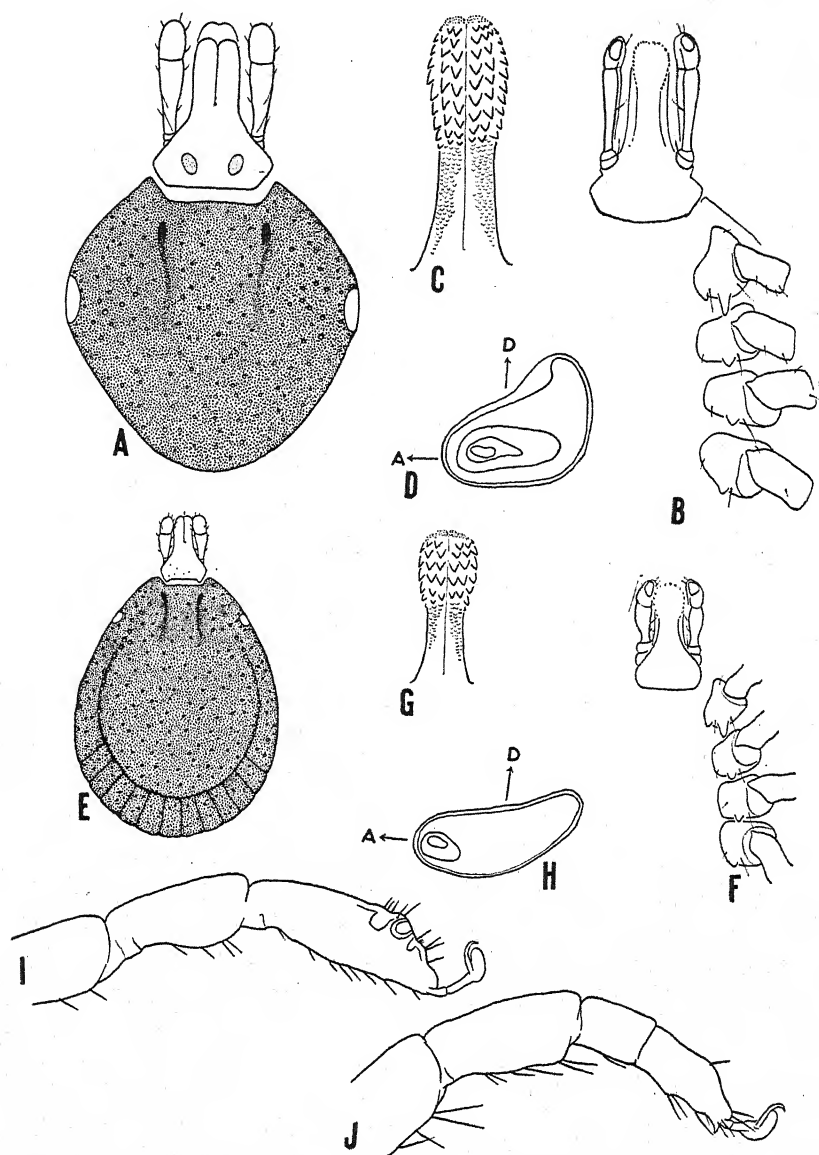


FIG. 11. *Amblyomma inornatum* (Banks). A. Capitulum and scutum of female. B. Capitulum (venter) and coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Capitulum (venter) and coxae of male. G. Hypostome of male. H. Spiracular plate of male. I. Leg I of female. J. Leg IV of female.

*Amblyomma avecolens* n. sp.

(Fig. 12)

Male and female unknown.

Nymph

*Body*: No unengorged specimens are available. Well-engorged specimens are very large, long-oval, slightly wider in front, measuring 6.5 long by 4.0.

*Scutum*: Length 1.02 to 1.14, width 1.08 to 1.11. Widest at the middle, scapulae pointed. Cervical grooves divergent backward, deep in front, and become valleys. Punctations conspicuous, deep, evenly distributed and contrasting strongly with the finely pebbled surface. Eyes small and flat.



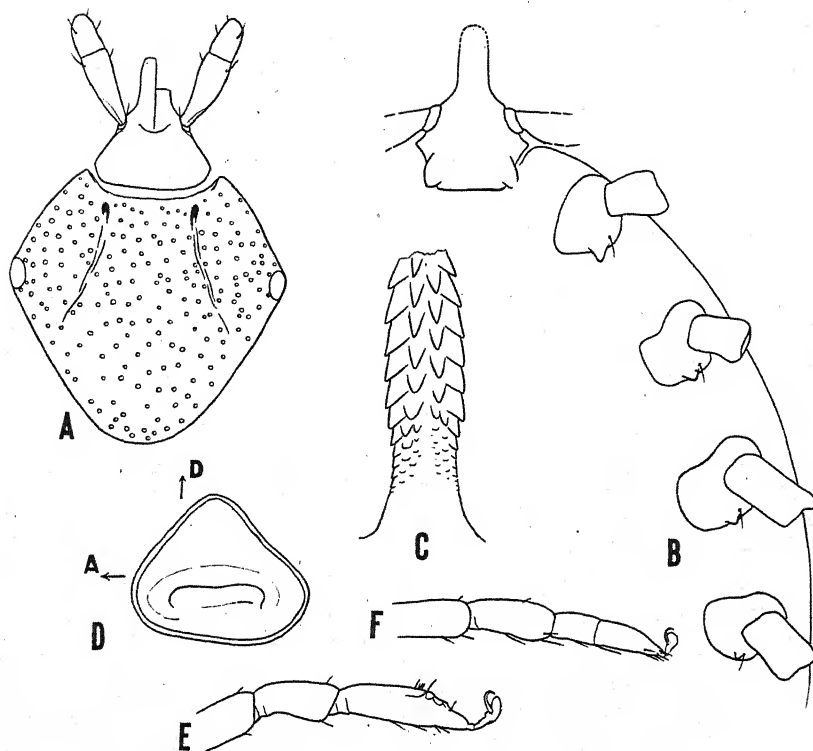


FIG. 12. *Amblyomma avecolens* n. sp. A. Capitulum and scutum of nymph. B. Capitulum (venter) and coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Leg I of nymph. F. Leg IV of nymph.

**Capitulum:** width of basis 0.45 to 0.48. (All capituli are mutilated and the length cannot be determined.) Sub-triangular, broadly rounded behind. Surface of basis and palpi finely pebbled, similar to the scutum. In ventral view basis is broad, with low lateral retrograde projections at the sides. Palpi long with a few fine hairs.

**Hypostome:** Long, with sides about parallel (the tip broken off). Dentition 2/2 and with files extending to near the base. Length (incomplete) 0.36.

**Legs:** Surface not smooth, suggesting the surface of the palpi. Tarsi long and tapering gradually; apical-ventral and sub-apical ventral spurs absent on all tarsi. Hairs few and short. Length of tarsus I, 0.54; metatarsus, 0.36. Length of tarsus IV, 0.45; metatarsus, 0.36.

**Coxae:** Small and with small, external spurs progressively smaller from I to IV, those on III and IV seen only with difficulty.

**Spiracular plate:** Sub-triangular, with the surface smooth, except at the macula, which is much depressed. Size 0.27 by 0.27.

Through the kindness of Dr. E. A. Chapin, Curator, Division of Insects, of the United States National Museum, we have had the opportunity to study three lots of nymphal ticks from birds, as listed below. The scutum is very characteristic and the bird host labels are of interest. These specimens are not easily referred to genus, but it appears reasonably certain that they are of a species of *Amblyomma*. The early stages of *Amblyomma* are not well known. Robinson (1926) omits them. Some of the specimens here described are very large and could not be of any known species in the United States, and the species of *Amblyomma* in Mexico are very little known.

**Holotype:** Nymph, 18707,\* 1 from neck of *Vireo griseus*, Aug. 5, 1929, 6 miles east of Sorita, Kennedy Co., Texas, deposited in the collections of the Rocky Mountain Laboratory.

\* Numerals refer to records of the Rocky Mountain Laboratory.

*Paratypes*: 18709, 1 from *Glyphorhynchus curvata*, May 3, 1926, Duck Run, British Honduras; 18702, 2 from *Xiphorhynchus* sp., Mar. 24, 1912, Panama Canal, deposited in the United States National Museum.

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## NOTES ON THE PUPAL DEVELOPMENT OF *STILBOMETOPA IMPRESSA* (DIPTERA: HIPPOBOSCIDAE)

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*Stilbometopa impressa* (Bigot), a common blood-sucking parasite of quail in California, has been reported only from *Lophortyx* spp. The present report is based on material collected entirely from *Lophortyx californica vallicola* and deals primarily with pupal development of the fly.

Knowledge of the biology of *Stilbometopa impressa* is of importance because of the fly's probable relationship to *Haemoproteus lophortyx*, a common malarial parasite of California quail. Thus far only the smaller fly *Lynchia hirsuta* Ferris has been demonstrated (O'Roke, 1930) to be a vector of this malaria. Work is now in progress in our laboratory to determine whether *S. impressa* is a vector of the malaria and as important in this regard as *L. hirsuta*.

### HISTORY

Studies on the biology of the bird hippoboscids received greater impetus when Sergeant and Sergeant (1906) demonstrated *Pseudolynchia canariensis* (Macquart) (= *Lynchia maura*) to be the transmitting host of *Haemoproteus columbae* of domestic pigeons. Their findings have been confirmed subsequently by many investigators in various parts of the world. O'Roke (1930) showed the fly, *Lynchia hirsuta* to be a vector of *Haemoproteus lophortyx*, and Herms and Kadner (1937) utilized *Lynchia fusca* as a vector of the quail haemoproteid. No other hippoboscid fly has been demonstrated as a transmitting agent of the various species of *Haemoproteus* in birds.

The biology of only a few bird hippoboscids has been investigated and *P. canariensis* has received the most attention. Sergeant and Sergeant (1907) stated that the larva, when deposited, is ovoid in shape, white, except at one pole, which is covered with a small black area containing six distinct branches. It becomes entirely black in one hour. These authors remarked that under the microscope very fine "hexagonal" lines appear on the surface of the pupa. Bishopp (1929) observed that the pupae of these flies are broadest posteriorly and slightly flattened dorso-ventrally. The surface is shiny and sculptured with "zigzag" lines except for the thickened posterior portion which is densely punctured and without lines. Drake and Jones (1930) reported that a single puparium of *P. canariensis* went through successive changes of yellow, light to dark brown and in the course of two or three hours, became strongly indurated, somewhat shiny and jet black in color. Coatney (1931) stated that the female fly deposits a single mature, whitish larva bearing a black post-abdominal spiracular plate and observed the same color changes of the puparium.

Huzimatu (1938) noted that the full grown larva of *Stenopteryx nipponica* Kishida, a parasite of martins in Japan, is globular in form and milky white in color. When the larva is deposited it is light brown in color, except the spiracular plate which is shining black. The integument afterwards becomes chitinized to form the

pupal case and there is a gradual darkening of the color until the puparium at last becomes shining black. From his observations (on two occasions) the color of the puparium turned from light brown to black in about two hours after deposition. He observed also, under the microscope, a network of fine lines.

In reporting the duration of pupal development, from the time the mature larva or pupa is deposited until the fly emerges, Sergeant and Sergeant (1907) in North Africa recorded 23 to 27 days (at a temperature of 42° C, which is the temperature of the plumage of the pigeon host); Adie (1915) in India, 31 to 36 days (but noted that some took 52 to 64 days to complete the pupal stage, and even longer as cold weather approached); Bedford (1924) in South Africa, 23 to 28 days; Bishopp, United States, 29 to 31 days (when the mean daily temperature was about 73° F—during the warmer part of the year development was accelerated slightly and the pupal period was longer under lower temperatures); Drake and Jones (1930), Iowa, (one larva deposited in September) 35 days; Coatney, also Iowa, 25 to 31 days (when kept at laboratory temperature—if the temperature was higher, the time was shortened a few days). Huzimatu reported that a single pupa of *Stenopteryx nipponica* produced an imago in 23 days. O'Roke observed that a single pupa of *Lynchia hirsuta* produced a fly in 31 days.

Hardenberg (1929) collected a number of *Stenopteryx hirundinis* Leach from swallows in Germany. He stated that the flies over-winter in the pupal state in the nest of the bird. He considered that there were two types of pupae: "summer" pupae which hatch out in about 22 days and "winter" pupae which do not hatch until the following year, not earlier than in April or May and usually not until the middle of June. Similar to Hardenberg's observations, Huzimatu noted pupae of *S. nipponica* in the abandoned nests of the birds after they had migrated southward and concluded that the imagoes would emerge in the spring of the next year when the birds came back to their old nests, thus also recognizing a distinction between summer and winter pupae.

Coatney observed that if the larva is tampered with before it has entirely pupated, in most cases it will fail to yield an imago. In observations on more than 50 larvae which had been handled prematurely, only one yielded an imago; 22 of these pupal cases were opened for examination and only two showed development which had been arrested early. Coatney found also that the flies usually deposited between 7 and 11 AM, seldom later in the day.

Coatney reported that of 221 imagoes of *P. canariensis* bred in the laboratory, 96 were males and 125 were females, concluding that the sexes are produced in about equal numbers. Hardenberg, in a series of 42 newly emerged *S. hirundinis*, counted 22 males and 20 females.

O'Roke, in his observations on *L. hirsuta* of quail in California, stated that no pupae or flies were taken apart from their hosts and no pupae were collected except those that were "oviposited"<sup>1</sup> in vials by captive flies.

Flies were taken in every month from June to October and hunters reported seeing *L. hirsuta* on quail during December and January.

<sup>1</sup> Undoubtedly means deposition of larva or pupa. These flies usually deposit their offspring in the pupal stage but may deposit them in the last phases of larval existence. Hence the deposited body may be referred to as a larva or pupa as the case may be.

## MATERIALS AND METHODS

Flies were collected in the field primarily by Charles Edmondson, John Laughlin and David M. Selleck of the California Division of Fish and Game and Roy W. Saarni of the U. S. Forest Service. The flies were obtained mainly from trapped wild quail (*Lophortyx californica vallicola*) in Orange, Riverside and San Benito Counties and from the San Joaquin Experimental Range at O'Neals, Madera County. In all these areas *Stilbometopa impressa* is a common parasite; a few specimens of *Lynchia hirsuta* were collected in Orange County in August, 1941, but none was observed on the birds from the other three localities.

Some of the flies were brought to the laboratory in a small screened cage containing a single quail. The cage containing the bird was taken into the field during trapping operations and all flies captured from quail were immediately placed into the small cage where they could seek a haven on their natural host. Where it was not feasible to bring the flies to the laboratory by this method they were shipped in shell vials approximately  $\frac{1}{2}$  inch in diameter and 2 inches deep. The aggressiveness of these flies made it necessary to ship only one per vial until Mr. Selleck experimented with placing a few quail feathers in each vial. Using this technique it was found that as many as 4 live flies could be shipped successfully. The feathers presumably served as a protection enabling the flies to escape each other's attacks. The flies were one or two days in transit.

As soon as the live flies were received at the laboratory they were placed in escape-proof cages with from one to seven quail. These cages were constructed with wire bottoms ( $\frac{1}{2}$ -inch mesh) with a collecting pan one inch below.

Most of the flies were obtained in August and September but this is not to be interpreted as the time of greatest abundance of *Stilbometopa* on the quail. The birds were trapped during these months as a part of other projects; collection of the flies was only incidental to other research.

Observations on *S. impressa* were begun in 1941 at the laboratory of the Los Angeles Wildlife Disease Research Station of the U. S. Fish and Wildlife Service. Detailed data on the pupal period were not sought in the earlier studies. Pupae were collected from the experiment cages at irregular intervals, usually about fortnightly.

In the summer of 1942 the studies were resumed at the State Game Farm in Chino, California, and continued in the fall at the Fish and Game laboratory in San Francisco. The pans of the cages containing birds and flies were examined several times daily. Pupae, or larvae, were placed, individually, in shell vials of the same type described above, loosely plugged with cotton. They were examined hourly during the first day to note color changes and then daily until the flies emerged. Throughout these experiments in the laboratory no attempt was made to regulate temperature which, on the average, was approximately 70° F.

## LIVABILITY OF ADULT FLIES

In the preliminary studies conducted in 1941 it was found that a number of flies placed in a cage with one or two quail yielded many pupae while an equal number of flies placed in a cage with seven quail produced very few, if any, pupae. Upon observation it was found that the birds would eat the flies. It was therefore assumed that in the limited confines of the cage with only one or two quail the flies were able

to avoid capture by the quail, while with many birds they had little chance to escape. The flies momentarily leave their hosts whenever the birds become excited, thus exposing themselves to capture. To test these points 25 flies were placed in an escape-proof cage with seven quail. Twenty-four hours later the birds were carefully removed and after extensive examination only a single fly was recovered. Similarly, 18 flies were placed in a cage with two quail. At examination three days later, 17 flies were recovered.

A colony of *S. impressa* was started by placing 47 adult flies (obtained from the wild quail) in an escape-proof cage with two quail. The flies were placed in the cage during August and September, 1941. Sixty-three pupae were collected at irregular intervals and of the 44 flies that emerged all, except the last one, were replaced into the same cage. During the experiment a total of 19 dead flies were collected in the pan beneath the cage. The flies seemed to steadily decrease in numbers (eaten by the birds?) until on March 20, 1942, no further flies could be found. (The last fly had been added to the cage on March 7, 1942.) A single fly emerged from a pupa after this date on April 13, 1942.

The longest period a fly remained alive off its host was five days.

#### DEPOSITION OF LARVAE

A single female was observed in the process of larvipositing. She supported herself on the first two pairs of legs. With one of the third pair of legs on the dorsal side of the body and the other on the ventral, the abdomen was stroked posteriorly as the larva slowly emerged through the vaginal opening. The entire process lasted over two minutes. Prouty and Coatney (1934) have reported a similar observation for *Pseudolynchia canariensis*. However, the author has observed females taken from wild quail which, in the excitement of being caught, seemed to larviposit almost instantaneously. It is questionable whether the straining procedure is not the exception rather than the rule.

The larva described in the preceding paragraph was creamy white when deposited but began to darken within a few seconds. Four others were observed during the course of these experiments. The larvae are soft, delicate, ovoid in shape,  $5.5 \times 4.5$  mm. At the posterior end is a black spiracular plate, 1 mm in diameter. They quickly darken, usually in less than one minute, although one was observed to remain white for at least seven minutes and since it eventually produced a fly it was considered within the limits of normal development.

All larvae in the experiment cages were found in the pan below the false bottom. The fly observed during larviposition was in the pan. Frequently female flies were observed in the pan. From this evidence it seems logical to assume that the flies normally leave the host to deposit the larvae.

In the laboratory there seemed to be no evidence of deposition of larvae by the flies at any particular time of day.

#### DEVELOPMENT OF PUPAE

From the preliminary studies on flies collected during 1941 it was evident that the puparium passes through several color phases before it becomes the characteristic shining black. In a number of cases careful observation was made of the time required for the pupal case to achieve its shining black color. Table 1 summarizes much of the data on the development of the pupae during the 1942 experiments.





variation than those collected at later dates, the variation increasing as winter progressed. The 5 pupae collected on January 26, 1942, produced flies on March 2, 3, 6, 13 and April 13.

From the flies collected off wild quail during 1942, 38 pupae were obtained, the first on August 25, 1942, the last on October 28, 1942. Only 19 flies emerged. In each case the exact date of deposition was noted.

The pupal development may last from 61 days (or less, as shown in the preliminary experiments) to as long as 162 days. The duration of this stage seems to increase (but in no definite progression) as the season advances. There is no definite demarcation of two types of pupae into summer and winter varieties as claimed for *Stenopteryx* spp. from swallows.

In emerging, the imago pushes open the anterior end of the puparium which splits along a definite line around the case about one-fifth of the length of the puparium. When the fly first emerges the wings are still folded within the wing sheath. It is very active on its legs and within half an hour the wings have spread so that the insect is able to fly.

Of 69 flies which emerged from pupae in the laboratory during the 1941 series of experiments, 39 were females and 30 were males. Of the 19 flies which emerged in the 1942 experiment, 8 were females and 11 were males.

#### SUMMARY

*Stilbometopa impressa*, a parasitic fly on *Lophortyx californica vallicola*, does not exist away from its host more than 5 days. The normal life expectancy of an individual fly was not determined. It runs the risk of being devoured by its host, particularly in confined or crowded quarters.

The larvae are creamy white, ovoid,  $5.5 \times 4.5$  mm, with a posterior spiracular plate 1 mm in diameter. The flies leave their host to deposit their larvae. Deposition may occur at any time during the day.

The puparium passes through a series of color phases until it is a shining black. This process may take as long as 16 hours. Imagoes emerge in from 61 to 162 days (in preliminary experiments as short a time as 29–43 days was recorded). The interval required for the pupal stage increases as winter advances but not in any definite progression.

Injury or contact with alcohol in the larval stage arrests development. Moistening with water does not prevent development. In a series of imagoes the sexes are produced in a ratio of approximately 1:1.

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## NOTES ON THE LUNGWORMS OF NORTH AMERICAN LEPORIDAE

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In a recent paper (Goble and Dougherty, 1943) the incidence and identity of the lungworms in Varying hares in eastern North America was discussed. The nematode described as *Synthetocaulus leporis* by Boughton (1932) was re-described and renamed *Protostrongylus boughtoni*. Four subspecies of *Lepus americanus* Erxleben were listed as hosts of this parasite in eastern United States and Canada. Incidences observed from 1939 through 1942 ranged from 11 per cent in animals from New Brunswick to 48 per cent in those from Dickinson township in the Adirondack region. It is the purpose of this article to report further observations on *P. boughtoni*, made in New York during 1943, and to comment on Scott's (1943) recent article on lungworms in North American leporids.

### OBSERVATIONS

Examination of the lungs of 31 hares (*Lepus americanus virginianus*) from Valcour Island (in Lake Champlain, near Plattsburg, N. Y.) revealed the presence of *Protostrongylus boughtoni* in 91 per cent of the adults (20 of 22) and 77 per cent of the juveniles (7 of 9). The infected juveniles were 5 weeks of age and older; the negatives were about 4 weeks old. The incidence of infection on Valcour Island is much greater than in other localities. This is probably due to the unusual environmental conditions prevailing there. Extensive predator destruction has resulted in an abnormally high game population in a habitat replete with the gastropod intermediate hosts of the parasite.

The same species of lungworm was also found in 6 out of 7 adult cottontails (*Sylvilagus floridanus mallurus*) and in 1 out of 3 juveniles. This is an unusual host record for this nematode, since it was not encountered in examinations of over 800 cottontails from other parts of New York state. The occurrence of more severe pathological conditions in the rabbits than those observed in hares also supports the view that the former is an abnormal host. Infection of cottontails in this locality is undoubtedly the result of the overlapping of their range with that of the Varying hares; intimate intermingling of these species is extremely rare in other localities.

### DISCUSSION

To our knowledge the only other records of lungworms in the cottontail group are those of Scott and Honess (1932) and Scott (1943). In the latter paper Scott described *Protostrongylus sylvilagi* (emend. nov. from *P. sylvilagii*, Scott, 1943) and reported its occurrence in the Black Hills cottontail (*Sylvilagus nuttalli grangeri*) and White-tailed jack rabbit (*Lepus townsendi campanius*) of Wyoming. Since this article was unavailable to us at the time *P. boughtoni* was redescribed and since Scott was apparently unaware of Boughton's *Synthetocaulus leporis*, it seems desirable to point out the differences between *P. sylvilagi* and *P. boughtoni*, which are the only lungworms so far reported for leporids in North America.

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The males of *P. sylvilagi* differ from those of *P. boughtoni* chiefly in the length of the spicules (156–180  $\mu$  in the former, 260–320  $\mu$  in the latter). There are probably other distinguishing characters, particularly in the gubernacular complex, which are not evident in Scott's photomicrographs. The females of *P. sylvilagi* differ from those of *P. boughtoni* in the lack of a prominent provagina (present in the latter) and in the length of the vagina (597–1116  $\mu$  in the former, 1900–2400  $\mu$  in the latter).

Scott distinguished *P. sylvilagi* from *P. pulmonalis* (syn. *P. commutatus*) on the basis of the length of the crura (51–84  $\mu$  in the former, 33  $\mu$  in the latter). Studies on the latter species within the last 20 years have shown the crural length to be 40–58  $\mu$  which is within the size range given for the parts in *P. sylvilagi*. Since these two species seem to resemble each other in most particulars, restudy of the New World form, to determine its status, is indicated.

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# PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL EUSTRONGYLIDES. VII. STUDIES UPON SURVIVAL AND METABOLISM IN STERILE SURROUNDINGS

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In a previous paper of this series (von Brand and Simpson, 1942) it was shown that larval *Eustrongylides* occurring normally in cysts of *Fundulus* can be kept alive a long time in vitro in various media, provided that conditions remain sterile throughout the whole course of the experiments. A continuation and amplification of these studies seemed indicated, since, with the exception of Glaser and Stoll's (1938) and Stoll's (1940) work on *Haemonchus* larvae, no data are known on the behavior of parasitic nematodes kept in vitro under sterile conditions. It becomes more evident, however, from year to year that the development of sterile media for the maintenance of parasitic worms will represent a starting point from which the physiology of these organisms can be studied with a much greater degree of reliability than was hitherto possible.

In the present paper the following points have been investigated: influence of pH, various carbohydrates and lowering of the temperature from 37° to 20° C on the survival. Also studied were the rates of consumption of oxygen, sugar, and glycogen under various conditions.

## MATERIAL AND METHODS

While in previous papers of this series the worm used was only tentatively designated as the larva of *Eustrongylides ignotus*, it can now be stated definitely that this identification was correct. The evidence will be presented in a later section.

As in the previous work the worms were isolated from cysts of *Fundulus heteroclitus*. The method of sterile extraction has been adequately described previously (von Brand and Simpson, 1942).

The media used in various series and their preparation will be discussed in the following sections. Only one point common to all series may be mentioned here, namely, that every isolated worm was kept in a separate tube.

## RESULTS

1. *Influence of pH upon the survival.*—The worms were isolated in test tubes of standard size each containing about 10 cc medium (Bacto-Peptide 0.5 per cent, NaCl 0.5 per cent and glucose 0.5 per cent). The tubes were closed with cotton plugs and fitted with parafilm which effectively prevents excessive evaporation. The medium had been sterilized by filtration (through Berkefeld "N" filters) rather than by autoclaving in order to avoid destruction of the sugar at the higher pH values. The medium was replaced every four weeks with the usual precautions to avoid contamination. A number of control tubes containing no worms was always carried along. These showed in general only very small changes from the original pH.

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<sup>1</sup> The author is indebted to the Elizabeth Thompson Science Fund for a grant towards the purchase of the respiration apparatus used in this investigation.

Column 3 of Table 1 represents the difference in pH between the tubes containing worms and the control tubes incubated at 37° C for identical periods. All pH measurements were carried out electrometrically.

A survey of the figures assembled in Table 1 makes it obvious that the optimal pH for survival in vitro lies around the neutral point. This is somewhat surprising since after extraction from the cyst and transfer to high temperature the worms are in a stage comparable to that of juvenile specimens beginning to establish themselves in the definitive host. As such, various species of herons may serve, and in these birds the worms become established in the glands of the fore stomach. The pH of the wall of the fore stomach, as determined by Mennega (1938) is strongly acid (pH 3.10) in starving herons and somewhat less acid (pH 4.14 to 5.75) in birds having received food from 45 minutes to 3½ hours prior to the determinations.

The figures shown in Table 1 are also a good proof for the extreme resistance of these helminths against the hydrogen ion, a fact already noted in previous studies concerning the rate of oxygen consumption at various pH (von Brand, 1943). The

TABLE 1.—Survival of larval *Eustrongylides ignotus* in sterile surroundings at various pH.  
Medium: Bacto-Peptone 0.5 per cent, NaCl 0.5 per cent, glucose 0.5 per cent.  
Temperature 37° C

Number of worms	pH at beginning	pH change after 4 weeks	Survival in days		
			Average	Minimum	Maximum
19	1.90	+ 0.20	21	7	48
18	3.77	+ 0.20	48	14	103
42	4.78	+ 0.10	62	20	115
40	5.74	- 0.09	84	14	179
27	7.16	- 0.28	114	32	225
23	7.94	- 1.08	84	36	151
25	9.40	- 1.35	50	2	97
31	11.30	.....	A few hours	..	...

survival even under fairly extreme conditions is still better than that usually found in other parasitic worms under the best possible conditions.

Of some interest are the changes in pH that occurred during the course of the experiments. In the extreme acid range a slight increase in pH was observed, while in the slightly acid or the alkaline tubes a lowering occurred. This drop was the more marked the more alkaline the initial reaction of the medium. The compensation point was not exactly determined but can be assumed to lie somewhere in the vicinity of pH 5. Similar observations are at hand also for other nematodes. Whitlock, Link and Leasure (1939) found that *Strongylus* shifts solutions that are strongly acid or alkaline in the direction of the pH occurring in their normal habitat (pH 6.35 to 7.91). Gettier (1942) observed a similar trend in tubes in which *Camallanus scabrae* were kept, the compensation point in this case being somewhere between pH 5 and 6. The experiments of these authors, however, were not conducted with sterile worms. It is therefore not clear whether these changes were due to the worms themselves or to the bacteria developing in the solutions used. The present observations are the first definite indication that such pH changes can be brought about by the worms themselves. The chemical mechanism underlying them will require further studies.

2. *Influence of various carbohydrates on the survival.*—In a previous paper (von Brand and Simpson, 1942) we have pointed out that the addition of 0.5 per cent glucose to various basic media considerably increased the survival in vitro under

sterile conditions. It was therefore deemed of interest to test whether other carbohydrates would have a similar influence.

The technique used was the same as that described in the previous section with the obvious difference that in most series instead of glucose a corresponding amount of one of the other carbohydrates was added to the medium and that a control series without any sugar was carried along.

The results of these series are summarized in Table 2. It appears that the only carbohydrates that increased the length of life of the worms in a significant manner were glucose, xylose and perhaps inulin. It is rather curious that mannose, fructose and maltose, all sugars that can be utilized by such a great number of various organisms, had no similar influence.

The pH changes in all series were quite similar. While, as pointed out above, the mechanism of the acidification is not yet known, it should be kept in mind that

TABLE 2.—Influence of carbohydrates on the survival of larval *Eustrongylides ignotus* in sterile surroundings. The carbohydrate concentration in the basic medium (*Bacto-Peptide* 1.0 per cent, *NaCl* 0.5 per cent) was in each case 0.5 per cent.  
Temperature 37° C

Carbohydrate	Number of worms	Extremes of survival in days	Average survival (M) and standard error (SE) in days	M carboh. - M contr.	CR*	Initial pH	pH change after 4 weeks
None (control) ..	30	12-118	65 ± 3.88	...	..	7.40	- 0.27
Cellobiose .....	29	22-105	61 ± 3.58	- 4	0.8	7.31	- 0.31
Xylose .....	27	45-125	84 ± 4.23	+ 19	3.3	7.20	- 0.34
Glucose .....	27	33-196	93 ± 7.32	+ 28	3.4	7.16	- 0.28
Mannose .....	24	43- 97	73 ± 3.17	+ 8	1.6	7.30	- 0.27
Laevulose .....	20	6-142	77 ± 7.51	+ 12	1.4	7.10	- 0.40
Maltose .....	30	14-203	71 ± 7.13	+ 6	0.7	7.20	- 0.20
Inulin .....	25	10-176	89 ± 8.39	+ 23	2.6	7.36	- 0.10

\* Critical ratio =  $\frac{M \text{ carboh.} - M \text{ contr.}}{\sqrt{SE(M \text{ carboh.})^2 + SE(M \text{ contr.})^2}}$ . If the figure in this column is greater than 2, the average survivals are significantly different.

these observations do not necessarily indicate that it is not connected with the carbohydrate metabolism. As will be shown in a following section, larvae having no access to sugar in the medium, which obviously would also be the case if a non-utilizable sugar only were present, metabolize rather considerable amounts of glycogen from their body reserves.

3. *Survival at 20° C.*—All the experiments described so far were conducted at body temperature. It appeared essential to study the survival also at a temperature that was comparable to that in which the normal intermediate host, *Fundulus*, lives. The series described in this section were therefore conducted in a constant temperature room at 20° C.

For various reasons the technique used was somewhat changed. It seemed inadvisable to close the tubes with cotton and parafilm since at this lower temperature molds have a tendency to grow through the cotton plug. The tubes were therefore closed with a sterile rubber stopper. Since the use of rubber stoppers (#5) in place of cotton plugs is more likely to permit contamination during removal and replacement of fluid it was decided to avoid the periodic opening of the tube that accompanies a renewal of medium. Instead, larger tubes (25 × 150 mm) were used and each worm was immersed in approximately 30 cc medium. This left a considerable air space above the fluid. While it must be assumed that the oxygen tension decreased



and the carbon dioxide tension increased during the long periods of survival, no signs of asphyxiation were observed.

Two series were carried out and their results are summarized in Table 3. The survival at 20° C is obviously excellent. During the first year of observation only 3 worms died, corresponding to a death rate of only 12 per cent. After 2 years most of the worms kept in Bacto-Broth had died but over 80 per cent of the larvae isolated into Bacto-Proteose Peptone survived.

These periods exceed by far all that have been described for any parasitic worm kept in vitro. The worms were perfectly quiescent and curled up at the bottom of the tubes just as they are in the cysts of *Fundulus*. After having lived for nearly two years in the constant temperature room in the dark they were exhibited for an evening to the Helminthological Society of Washington. During this time they were exposed to a somewhat higher room temperature and to strong light, which caused the worms to become quite active, just as active in fact as freshly isolated specimens. The series are not yet completed and will be observed further. Unfortunately no suitable definitive host is available; it would have been of great interest to test whether these larvae still retain their infectivity.

TABLE 3.—*Survival of larval Eustrongylides ignotus at 20° C in sterile media. Series A: Bacto-Broth 1.6 per cent, NaCl 0.85 per cent, glucose 0.5 per cent. Series B: Bacto-Proteose Peptone 1.0 per cent, NaCl 0.85 per cent, glucose 0.5 per cent*

Number of worms at beginning	Number of worms living after specified month				
	6	12	18	24	30
Series A: 14 .....	14	13	13	3	2
Series B: 12 .....	12	10	10	10	7

4. *Sugar, glycogen and oxygen consumption, in vitro.*—In order to study quantitatively some phases of the metabolism of the larvae in vitro, several facts had to be considered that necessitated some changes in the experimental procedure. If sugar was desired in the medium its concentration was kept at about 200 mgm per cent rather than at 500 mgm per cent because the method used for its determination (Hagedorn and Jensen, 1923) is more easily carried out in the former range. The amount of medium introduced into each tube was exactly 10 cc instead of the approximate amounts used in the other series. The medium was distributed into the tubes immediately after Berkefeld filtration from a filter flask fitted with a sterilized dispensing apparatus, the essential feature of which was a 50-cc burette, graduated to 0.1 cc. The test tubes were closed with sterile rubber stoppers in order to avoid any evaporation. While cotton plugs covered with parafilm are quite sufficient to prevent any large degree of evaporation they do not guarantee an exact maintenance of the volume which was of paramount importance for the intended experiments. Half of the series were conducted at 37° C and half at 20° C.

After the intervals stated in Table 5 both the control tubes without worms and the experimental tubes were opened for the chemical determinations. Before the samples for the latter were taken out, however, samples were withdrawn in order to test whether the sterility of the medium had been maintained. These bacteriological controls were carried out as follows: By means of individual sterile pipettes fitted with rubber bulbs, a sample was removed from each tube and a portion inoculated

into a tube of glucose broth and the remainder into a tube of sodium thioglycollate medium. The amount of inoculum was approximately 0.5 cc. These tubes were incubated at 37° C for 48 hours or longer. All tubes proved to be sterile.

Immediately after the bacteriological sampling the sugar content of each tube was determined. It was found that the changes occurring in the control tubes during the periods of incubation were negligible and entirely within the normal range of error of the method. The greater changes observed in the tubes that contained worms could therefore safely be attributed to the metabolism of the latter.

After the sugar samples had been withdrawn the worms were removed from the tubes and a part of the remaining medium was used for a pH determination.

The worms were weighed individually and their oxygen consumption was determined in a Warburg apparatus, using the same technique as described in previous papers (von Brand, 1942, 1943). These determinations were not carried out with the single worm specimens, since the manometer readings would then have been too small to be accurate, but two or three worms were used together. The fluid introduced into the vessels had been taken from the experimental tubes in which the worms

TABLE 4.—*Glycogen content and oxygen consumption of freshly isolated larvae of Eustrongylides ignotus. Average figures*

Series	Number of worms	Average weight of single worm mgm	Medium*	Temp. (° C)	Oxygen consumption in cmm per 1 gm and 1 hour	Glycogen in per cent of fresh weight
Control 1 ...	30	80	A	37	166	8.24
" 2 ...	32	74	B	37	157	8.51
" 3 ...	36	88	C	37	148	...
" 4 ...	16	73	A	25	82	...
" 5 ...	15	60	B	25	95	...
" 6 ...	41	76	C	24	67	8.59

\* A: Bacto-Broth 0.8 per cent, NaCl 0.6 per cent, glucose 0.2 per cent.

B: NaCl 0.8 per cent, glucose 0.2 per cent.

C: NaCl 0.85 per cent.

had lived previously. The oxygen determinations with those worms that had been kept at 37° C were carried out at that same temperature, those with the worms of the 20° C group were performed at 24 or 25° C, since the waterbath had to have a somewhat higher temperature than the surrounding air in order to function properly. In a previous paper (von Brand, 1943) it has been shown that the temperature coefficient in this range is 3.55 and this figure was used in calculating the actual oxygen consumption during the experimental period at 20° C. Most of the oxygen determinations were carried out for periods of 4 hours with half-hour readings in the case of the 37° series and hourly readings in that of the 20° group.

These determinations were followed by determinations of the glycogen content of the worms according to the micro-modification of Pflüger's method as described by von Brand (1936).

It was unfortunately not possible to determine either the weight or the rate of oxygen consumption of these worms at the start of the experiments since during such procedure bacterial contamination would have been unavoidable. Although some changes in weight must undoubtedly have occurred it does not appear likely that they were very pronounced. The average weight of the worms at the termination of the experiments was about the same as that of worms recently isolated from fish, as a comparison of the figures in Tables 4 and 5 shows. In order to get an initial value for the oxygen consumption and the glycogen content, series of freshly

TABLE 5.—Glycogen content and oxygen consumption of larval *Eustrongylides ignotus* kept *in vitro* under sterile conditions for specified periods and sugar content of the medium. Average figures

Series	Number of worms	Average weight of single worm	Temperature	Medium*	In vitro period in days	Sugar content at end of in vitro period		Oxygen consumption		Glycogen per cent of fresh weight
						Control tubes	Worm tubes	Temp.	mm O <sub>2</sub> /1 gm./1 hr.	
		mgm	° C					° C		
1	16	66	37	A	23	22.4	21.0	37	86	7.00
2	18	84	37	A	39	22.3	20.4	37	92	5.08
3	22	91	37	B	24	20.2	18.2	37	97	6.41
4	18	89	37	C	21	...	...	37	108	6.14
5	12	85	37	C	39	...	...	37	79	4.75
6	10	65	20	A	130	21.1	20.4	24	56	9.22
7	12	101	20	A	264	21.4	20.4	25	59	6.23
8	10	93	20	B	108	20.4	17.8	25	51	8.29
9	16	93	20	B	250	20.8	16.7	25	58	6.34
10	16	82	20	C	146	...	...	24	38	5.53
11	16	71	20	C	256	...	...	25	47	2.86

\* A: Bacto-Broth 0.8 per cent, NaCl 0.6 per cent, glucose 0.2 per cent.

B: NaCl 0.8 per cent, glucose 0.2 per cent.

C: NaCl 0.85 per cent.

isolated worms were used in which the oxygen consumption was determined at the same temperatures and in the same media that were used for the experimental lots. The glycogen figures of the initial series were very similar and their mean value, 8.45 per cent, is thought therefore to be representative of the initial glycogen content of the experimental worms.

The results of the experimental series are summarized in Tables 5 and 6, the former table being essentially a summary of the analytical data, the latter showing the results of the experiments calculated per 1 gm and 1 day.

If a comparison is made of the rates of oxygen consumption of the freshly isolated and the experimental worms (Tables 4 and 5) it becomes obvious that they are smaller in the latter. In a previous paper (von Brand, 1942) it was shown that the oxygen consumption declines rather rapidly after removal of the worms from the fish. A comparison of the rates of the series kept at the same temperature and in the same medium for various periods, at 20° for more than 8 months, shows that they remained quite constant. Only the latter figures were therefore used for the calculation of the average oxygen consumption (Table 6). It is believed that this neglect of the initial value introduced no great error.

TABLE 6.—Data of Table 5 calculated per 1 gm worm and 1 day. Mean values for sugar, glycogen and oxygen consumption

Series	Sugar consumed		Total carbohydrate consumed per day	Oxygen required for total oxidation of carbohydrates	Oxygen consumed per day	Oxygen required for total oxidation in per cent of consumed O <sub>2</sub>
	From medium	Derived from glycogen				
	mgm	mgm	mgm	mgm	mgm	
1	0.92	0.70	1.62	1.72	2.94	58
2	0.55	0.96	1.51	1.61	3.16	51
3	0.92	0.94	1.86	1.98	3.33	59
4	...	1.22	1.22	1.30	3.70	28
5	...	1.05	1.05	1.12	2.72	24
6	0.08	+ 0.06	0.02	0.02	1.15	2
7	0.04	0.09	0.13	0.14	1.22	11
8	0.26	0.02	0.28	0.30	1.05	29
9	0.18	0.09	0.27	0.29	1.33	22
10	...	0.22	0.22	0.23	0.78	29
11	...	0.24	0.24	0.26	0.86	30

It is quite apparent that the worms are capable of utilizing glucose that is present in the medium. A study of the analytical data from the series kept in the same medium for various lengths of time indicates however that this consumption takes place primarily during the first weeks. Later on it seems to have ceased entirely in experiments 2 and 7, but it was still quite pronounced in series 9. As a consequence the overall daily sugar consumption as computed in table 6 appears usually smaller in the longer lasting experiments.

In the 37° series (series 1 to 3) no difference in sugar consumption from the medium occurs whether the medium contained peptone or not, in the 20° series, however, it was considerably greater in the latter case.

In all series, with the exception of No. 6, some glycogen disappeared from the body. It is characteristic that in the series kept in the same medium for various periods at the same temperature relatively more glycogen disappeared during the longer incubation periods than during the shorter ones. Consequently the overall daily glycogen consumption is greater during the former—exactly the opposite of the case where sugar is present in the medium. Both phenomena are of course connected. At times in which the worms consume carbohydrate from the medium less glycogen has to be mobilized from the body reserves in order to cover the carbohydrate needs of the organisms. In cases where no sugar was present at all in the medium the glycogen consumption was the greatest (series 3 and 4, 10 and 11) and, what is especially characteristic, the daily consumption was about constant regardless of the duration of the experimental periods, depending in its intensity only on the temperature.

An actual increase in glycogen occurred only in series 6. It was relatively small and may not be real. The worms of this lot may have had initially a glycogen content surpassing the average.

The total carbohydrate consumption (sugar derived from glycogen and, where present, from the medium) is obviously a composite between the different behavior of the two fractions. It is noteworthy that the differences between the total daily carbohydrate consumptions that exist between identical series carried on for different periods are small and obviously within reasonable limits of error. This indicates that the carbohydrate consumption remained stable at 37° for a minimum of 5 to 6 weeks and at 20° for at least 8 months. This should mean that the worms remained quite normal outside their hosts at least for these periods.

An attempt has been made (Table 6) to determine whether the carbohydrate metabolism is an important part, quantitatively, of the total metabolism by calculating how much of the daily oxygen consumption is used for sugar oxidation. Several difficulties arise that make such a calculation somewhat insecure and the figures assembled in the last column of table 6 may therefore be open to future readjustment. In the first instance as has already been explained, the oxygen consumption figures are derived only from a final determination at the end of the experiments and one may doubt whether they can be used for an average of the whole period. In the second place it has been found previously (von Brand, 1942), that, at least in freshly isolated worms, the respiratory quotient is slightly above 1. This indicates probably that some incomplete oxidations are going on even at the oxygen tension of the atmospheric air. This may even be more pronounced than appears on the face of it. If, as the present figures indicate, the metabolism of the larvae in question is not a pure



carbohydrate metabolism the purely oxidative respiratory quotient would obviously lie below 1 and the increase of the overall quotient above 1 would indicate a greater participation of partial oxidations or fermentations. Provided that the mother substance which they attack is carbohydrate, as happens in many other cases, the oxygen actually used in the carbohydrate breakdown would be less than if one assumes total oxidation of the sugar.

Keeping these limitations in mind we may try to evaluate the data. It would appear then that in media containing sugar and kept at 37° about half of the total oxygen is used in the carbohydrate metabolism (series 1, 2 and 3), while in those lacking sugar (series 4 and 5) the amount is only about 25 per cent. The situation is different in the 20° series. In no case was more than 30 per cent of the consumed oxygen attributable to the carbohydrate metabolism. Only an exceedingly small amount of it could be referred to sugar oxidation if the medium contained peptone (series 6 and 7), perhaps an indication that this latter substance can also be utilized.

It is believed that the figures, despite their admitted insecurity, are an indication that the metabolic processes are different at low and high temperatures. This result is perhaps not too surprising. Previous work (von Brand, 1942) has indicated that the worm must be able to cover all its oxygen needs in the fish; once it is in the final host it may have more difficulty in doing so. It is, however, a general feature that with restricted oxygen supply the carbohydrate metabolism comes more and more into the foreground. It would appear possible that the mechanism that brings about the transition from one metabolic type to the other is the temperature, since the temperature of both hosts is characteristically different. Another indication that the metabolic processes may be different at different temperatures has already been found previously (von Brand, 1943). It was shown that the temperature coefficient of the oxygen consumption is 3.55 at low temperatures (up to 27° C) while above that limit it is only 1.48.

5. *Identification of the larvae.*—*Eustrongylides* larvae of different species are very similar and a specific identification cannot be given with absolute certainty. Dr. B. C. Chitwood, to whom the worms in question were submitted several years ago, identified them therefore only tentatively as *Eustrongylides ignotus*.

It now happened that one of the larvae, belonging to series 2 described in the last section, molted in vitro into an adult male. The specimen was preserved in formalin and sent to the Bureau of Animal Industry where it was studied by Mr. J. T. Lucker. He reports that, although minor differences in the arrangement of the papillae are present compared with the original description, it is *Eustrongylides ignotus*. The papillae correspond exactly to those of specimens collected by Cram (1933) from *Nycticorax nycticorax hoactli* which had been identified by her as *Eustrongylides ignotus*.

The worm was then sectioned in order to find out whether the reproductive organs had matured. However no definite signs of sperm formation was found; it was apparently still a juvenile specimen.

No definite reasons can be given why this particular animal molted while the far more than 1,000 specimens that have been kept isolated during the present and previous studies did not do so. It may be pointed out that, contrary to most other experiments, the tubes of this series were closed by means of rubber-stoppers and had a sugar concentration of only 0.2 per cent. The temperature, as in most other series,

was 37° C. Whether a lowered oxygen tension or an increased carbon dioxide tension may have played a role will have to be decided by further studies. It is of interest in this connection, that Stoll (1940) found that the first parasitic ecdysis of *Haemonchus contortus* is definitely favored by a lowered oxygen tension.

## SUMMARY

1. All experiments were performed under strictly sterile conditions.
2. The larvae of *Eustrongylides ignotus* survive longest when the medium has a pH around the neutral point, but they survive relatively well also in quite acid and alkaline surroundings.
3. In very acid surroundings the worms shift the pH of the medium slightly towards the alkaline side. In but slightly acid or alkaline media they are responsible for an acidification.
4. The survival in vitro is favored if the medium contains either xylose, glucose or perhaps inulin. No significant influence was found with cellobiose, fructose, mannose or maltose.
5. The survival at 20° C is much longer than at 37°. The worms lived in the former temperature over 2½ years in vitro.
6. Quantitative data are presented concerning the sugar, glycogen and oxygen consumption for worms kept in vitro at 37° for periods up to 5 weeks and at 20° for periods up to 8 months. It is pointed out that at the former temperature relatively more oxygen seems to be used in the carbohydrate metabolism than in the latter.
7. Due to the fact that one specimen molted in vitro into an adult male a specific identification of the worms became possible. The previous tentative identification as the larvae of *Eustrongylides ignotus* could be confirmed.

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## AMERICAN SOCIETY OF PARASITOLOGISTS

### PRELIMINARY ANNOUNCEMENT OF THE NINETEENTH ANNUAL MEETING

*Monday, Tuesday, and Wednesday, September 11-13, 1944, Cleveland, Ohio*

In accordance with the action of Council, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Cleveland, Ohio, from September 11 to 13, 1944.

A call for papers has already been sent to all members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the Secretary not later than June 15, 1944. Members are hereby reminded of this date, and are requested to submit papers promptly.

So far as can be anticipated, the program will be arranged as in previous years, although some changes may be necessary because of circumstances resulting from the War. In the event that war conditions in September make a general meeting impractical, it is possible that regional meetings may be substituted or that the proposed meeting will have to be postponed or cancelled.

JAMES T. CULBERTSON,  
*Secretary.*

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## INFLUENCE OF THE DIET OF BLATTIDAE ON SOME OF THEIR INTESTINAL PROTOZOA<sup>1</sup>

SISTER JOSEPH MARIE ARMER

### INTRODUCTION

Many studies have been made with respect to the occurrence, cultivation, cytology, and life cycles of the intestinal protozoa of the common Blattidae, but only one investigation, that of Sassuchin (1931), is in any way concerned with the influence of the food of the host on the protozoa inhabiting its intestine. The effects of the diet of other invertebrate hosts upon their intestinal fauna have also received but little attention, except for a few interesting investigations, such as those on termites made by Cleveland (1925a and b) and Lund (1930). Data relative to the influence of food supply on free-living protozoa are given in papers by Adolph (1929) and Harding (1937a). Similar studies on the intestinal protozoa of various vertebrate hosts are discussed in the work of Hegner (1923 and 1937), Becker (1932 and 1937), MacLennan (1934), Becker and Morehouse (1935, 1936, and 1937), and Hegner and Eskridge (1937a and b).

Since many of the intestinal protozoa of cockroaches live directly on the food eaten by the host, a study of the influence of its diet on the parasites was deemed to be of interest. Roaches are especially favorable hosts for such an investigation because they harbor a great variety of intestinal protozoa. The forms to which special attention was paid in this work were *Nyctotherus ovalis*, *Endamoeba blattae*,<sup>2</sup> *Endolimax blattae*, *Lophomonas striata*, and *Lophomonas blattarum*. The principal points considered were:

- (1) Persistence of infection with the various parasites when the hosts were kept under the following conditions: starvation, high-carbohydrate, high-fat, and high-protein diet.
- (2) A qualitative and semi-quantitative study, with the help of staining methods, on the influence of the various diets on the polysaccharide and fat reserves of the parasites.
- (3) A study of the influence of the foregoing factors on the size of *N. ovalis*.

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<sup>1</sup> A contribution from the Department of Biology, the Catholic University of America, Washington, D. C. This paper, prepared under the direction of Dr. Theodor von Brand, is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to acknowledge her indebtedness and sincere gratitude to Dr. Theodor von Brand for his untiring efforts and constant interest in the direction of this problem, and to Dr. E. G. Reinhard for his encouragement and helpful suggestions throughout the work.

<sup>2</sup> In harmony with the suggestion of Morris, the amoebae of intermediate size, corresponding to *E. thomsoni* were counted as belonging to *E. blattae*.



(4) A quantitative study of the influence of the above factors on the numbers of *N. ovalis* persisting or developing.

#### MATERIALS AND METHODS

The roaches were collected at several localities in Washington, D. C. In the initial studies to determine the percentage of infection, three species, *Periplaneta americana* (L.), *Blatta orientalis* L., and *Blattella germanica* (L.), were examined. However, since these preliminary observations showed that *P. americana* had a higher incidence of infection than either of the others and occurred in larger numbers in this region, it was used exclusively in subsequent investigations. Other factors which made it especially favorable for these studies were its large size, its hardiness, and its ability to endure starvation for long periods.

Preliminary observations revealed no appreciable difference between the percentage of infection in full grown animals and that in the developmental stages. Consequently, both nymphs and adults were employed in the experimental work. Although nematodes were found in the intestines of many of the roaches, there was no indication that they had any influence on the protozoan population.

#### *Persistence of Infection*

For these experiments, groups of four hundred roaches were maintained under each of the following conditions:

- (1) Starvation.
- (2) High-carbohydrate diet, consisting of cornmeal.
- (3) High-protein diet provided by "Protein Milk Powder" having an approximate composition of: (a) Protein, 37.0%; (b) Milk fat, 26.5%; (c) Lactose, 26.0%; (d) Minerals (ash), 6.0%; (e) Lactic acid, 3.0%; (f) Calcium, 1.7%; (g) Moisture, 1.5%; (h) Phosphorus, 1.0%; (i) Iron, 0.001%.
- (4) High-fat diet, composed of vegetable fat.

All of the experimental animals were provided with an ample supply of water.

Every series of four hundred roaches, except in the case of the starved animals, was divided into two lots of two hundred individuals each. The members of one group were kept in batches of about forty in glass jars of one gallon capacity, thus giving an opportunity to study the infections in a population. Those of the other group were placed individually in half-pint milk bottles in order to avoid the possibility of new infections becoming established. In the starvation experiment, it was found necessary to keep all of the animals individually because they became cannibalistic when starved.

The initial infection of the hosts was determined for roaches obtained from different localities and at various seasons of the year. These determinations were made at the times when the different series were set up. In making examinations for qualitative studies, the hind-gut was removed, macerated in a drop of 0.7% saline on a glass slide, and examined with a compound microscope. The intestinal contents of forty individuals from the mass and individual cultures of the various series of test animals were examined in this way at the end of two weeks and the kinds of protozoan parasites present were noted. Other groups, of forty roaches each, were studied in the same way at the end of four-, six-, eight-, and ten-week periods.

*Studies on Polysaccharide and Fat Reserves of Parasites*

For the studies on the polysaccharide reserves of the parasites, permanent slides were prepared from cover-glass smears of the intestinal contents fixed with Carnoy's fixative and stained by Bauer's (1933) modification of the Feulgen technique. Controls were made by using smears of the intestinal contents of the same host and staining them after one hour's digestion with filtered saliva.

Several hundred slides were prepared in the manner outlined above and later used (1) in making observations for the presence of glycogen in the various parasites, (2) in semi-quantitative studies on the amount of paraglycogen present in *Nyctotherus ovalis*, and (3) in determining the influence of the different diets on the size of *N. ovalis*.

In making the semi-quantitative studies on *N. ovalis*, four standards having varying amounts of paraglycogen were selected as a basis for comparison. From twenty-five to one hundred individuals, depending on the number present in the slides, were observed for each diet initially, after four weeks, and again after eight weeks, in order to determine the percentage of animals that corresponded to each of the standards used.

The influence of the various diets on the size of *N. ovalis* was studied by measuring the length and width of the same animals that were used in making the paraglycogen determinations. A micrometer eye-piece was employed for these measurements.

Since it was found difficult to get the smears to adhere to cover-glasses when formalin was used as a fixative, permanent slides were not made for investigations on fat, but the routine slides were stained directly with Sudan III.

*Number of N. ovalis per Host*

In studying quantitatively the influence of the various diets on the number of persisting parasites, *N. ovalis* alone was considered, since its size and regular occurrence make it best suited for such observations. In order to have all of the animals as nearly uniform in size as possible, only adult roaches or large nymphs were used. The feeding was carried on as in the experiments described above, except that all of the specimens used were kept in individual bottles. The initial number of parasites present was determined and subsequent counts were made after periods of two and four weeks, respectively.

The preparations for counting were made by dissecting the host animals, removing the entire hind-gut, macerating it completely in a drop of 0.7% saline on a glass slide, and covering it with a rectangular cover-glass (40 × 22 mm). In most cases a total count was made, using the 16-mm objective and moving the slide by means of a mechanical stage. However, when very large numbers of parasites were encountered, a slide marked off in centimeter squares was employed; several centimeter areas were counted and the total number of parasites present was then calculated.

## EXPERIMENTAL DATA

*Persistence of Infection*

The purpose of the first experiments was to determine how long the parasitic protozoa under consideration would survive in each of the series described above.

The complete results obtained for the mass cultures are given in Table 1, while those for the individual cultures are shown in Table 2.

The percentage of infection with *N. ovalis* remained at about the same level, regardless of starvation or the kind of food supplied. Results obtained with the mass and individual cultures were essentially identical, indicating that the maintenance of a high infection rate was not due to reinfection.

On the other hand, variations in host diet did influence, more or less markedly, the persistence of infection with the flagellates and amoebae. *Lophomonas blattarum* was entirely eliminated by starvation, as well as by a diet high in protein; it was also practically eliminated by the high-fat diet. The high-carbohydrate diet was the most favorable for this species, since the infection, although becoming lighter, persisted throughout the ten weeks of the experiment.

*Lophomonas striata* also disappeared from some hosts on high-fat and high-protein diets, but it was not influenced as much by starvation as was *L. blattarum*. The former parasite, like the latter, persisted longer and in a higher percentage of host animals when the diet was high in carbohydrates, but at the end of the experi-

TABLE 1.—Persistence of parasites when hosts were kept in mass cultures: (a) at start; (b) 2 wks.; (c) 4 wks.; (d) 6 wks.; (e) 8 wks.; and (f) 10 wks. after isolation

Parasite	High-carbohydrate diet % of roaches infected						High-protein diet % of roaches infected						High-fat diet % of roaches infected					
	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)
<i>N. ovalis</i> .....	62	77	77	—	60	72	76	65	58	50	53	55	76	47	60	65	62	42
<i>L. striata</i> .....	40	52	42	—	30	25	12	7	2	0	2	0	12	0	0	2	2	2
<i>L. blattarum</i> .....	35	17	7	—	25	22	7	5	0	0	0	0	7	7	2	7	2	2
<i>Endamoeba blat.</i> ...	50	72	45	—	45	47	37	22	5	0	5	15	37	5	10	7	25	15
<i>Endolimax blat.</i> ...	48	70	42	—	35	30	37	15	5	30	25	40	37	2	2	15	20	22
Small flag. ....	78	90	97	—	90	85	100	82	85	87	75	70	100	87	85	92	82	67

ment the percentage of infection for *L. striata* was higher than it was for *L. blattarum*.

The amoebae decreased decidedly under starvation, *Endolimax blattae* being entirely eliminated after eight weeks. Again, on the high-carbohydrate diet, the infection with both species persisted throughout the experiment in a relatively large number of individuals. On the high-fat and high-protein diets, *Endamoeba blattae* showed a decrease in infection rate, but was not entirely eliminated. The results obtained with the high-fat and high-protein diets on *Endolimax blattae* were somewhat irregular. With both of these diets, the infection rate declined very definitely after two to four weeks but became relatively heavy again after four to six weeks. There are several possible explanations for these results. In the first place, the infection rate in the roaches used might not have been as uniform as was supposed. However, this does not seem very likely because similar results were obtained for two different diets with both the mass and the individual cultures. Another possibility is that the infection became very light during the first few weeks of the experiment and, as a consequence, the number of individuals present was so small that they were overlooked when the intestinal contents were examined. Those amoebae which persisted possibly represented adaptable strains which by subsequent division produced the heavy infections present later in the experiments.

Besides the five species of protozoa already discussed, a number of species of very small flagellates were frequently present in the hind-gut of *Periplaneta americana*.

TABLE 2.—Persistence of parasites when hosts were kept in individual cultures: (a) at start; (b) 2 wks.; (c) 4 wks.; (d) 6 wks.; (e) 8 wks.; and (f) 10 wks. after isolation

Parasite	Starvation % of roaches infected						High-carbohydrate diet % of roaches infected						High-protein diet % of roaches infected						High-fat diet % of roaches infected					
	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)
<i>N. ovalis</i> .....	62	62	55	85	72	64	70	70	57	75	75	70	62	72	57	70	65	65	62	57	70	62	70	77
<i>L. striata</i> .....	40	40	37	22	10	25	24	37	24	22	22	27	15	15	5	2	2	0	15	5	2	7	0	5
<i>L. blattarum</i> .....	35	20	5	0	0	0	28	20	24	20	15	12	7	10	0	0	0	0	7	2	10	0	0	2
<i>Endamoeba blat.</i> .....	50	42	17	5	2	4	44	55	46	53	43	32	40	42	10	17	17	15	40	15	22	7	10	17
<i>Endolimax blat.</i> .....	48	50	0	10	0	0	42	37	26	42	35	20	45	30	17	36	62	32	45	7	5	2	45	40
Small flag. ....	78	82	87	97	90	96	84	100	90	90	87	90	97	92	82	82	92	90	97	87	92	85	92	82



They were not studied in detail, but as can be seen from the tables, the various diets did not appreciably influence the persistence of these organisms as a whole.

In general, there was very little difference in the results obtained for the mass and individual cultures, indicating that the transfer of parasites from one host to another did not play an important rôle in the persistence of the parasites.

#### *Carbohydrate Reserves*

Carbohydrate reserves which are stored in the form of polysaccharides have been demonstrated in many protozoa, both in free-living and in parasitic forms (for a detailed list see von Brand, 1935). There are, however, to the writer's knowledge few studies in which the influence of the diet of a host animal on the carbohydrate reserves of its intestinal protozoa has been considered. In the present investigation, the glycogen and paraglycogen content of the five most common intestinal protozoa of the cockroach was studied qualitatively, while a semi-quantitative study was carried out on *N. ovalis*. Only the motile forms were studied, cysts occurring too sporadically for detailed study.

In general, it may be said that, except for *N. ovalis*, the observed species of parasites showed little, if any, storage of glycogen. *Endamoeba blattae* and *Endolimax blattae* ingested large numbers of starch grains when the host was fed on a high-carbohydrate diet; but, surprisingly, only a very little glycogen was found and that was diffused in the cytoplasm. *Lophomonas striata* has no cytostome and contains no solid food. Its food probably consists of dissolved material which is taken in by osmosis through the body surface. It ordinarily contained only traces of glycogen but the amount present increased on a high-carbohydrate diet, becoming especially abundant along the numerous rod-like structures which characterize the species. *Lophomonas blattarum* contained numerous food particles, many of which were starch grains, but the staining methods employed showed no trace of glycogen as a storage product.

*N. ovalis*, on the other hand, contains a very large amount of glycogenous material which is apparently paraglycogen because it is relatively insoluble in water. This reserve carbohydrate occurs in the form of granules which show considerable variation in shape and size but usually have the form of a more or less rounded disc. Between the anterior end of the body and the macronucleus, these granules occur in very large numbers forming a bean-shaped mass. Although the granules are most numerous in this anterior region, typically some are also scattered throughout the rest of the cell. As will be seen from the semi-quantitative studies, this ciliate, under certain conditions, becomes almost entirely filled with the paraglycogen granules.

As previously stated, four standards (Fig. 1) based upon four selected individuals, were established as references. The first contains only a small number of scattered paraglycogen granules; the second has the bean-shaped mass at the anterior end packed with granules and also a few scattered granules; the third has relatively large numbers of granules in the various body regions, while the fourth is entirely filled with the glycogenous granules.

Significant results (Table 3) were obtained in starved animals and in roaches given a high-carbohydrate diet. In the former, parasites with much paraglycogen had markedly decreased after four weeks, but there was no further decrease when

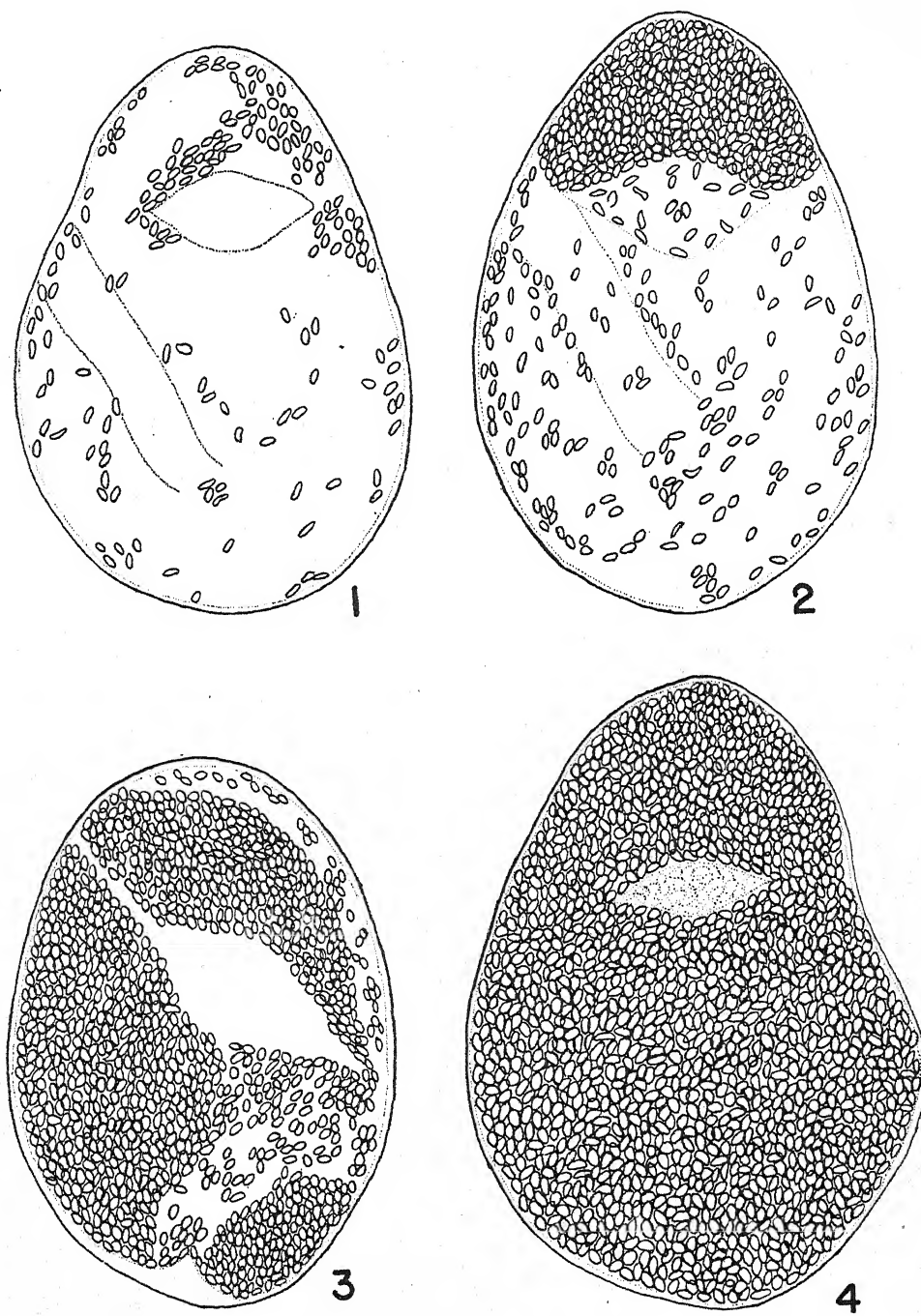


FIG. 1. Polysaccharide storage in *Nyctotherus ovalis*. (The granules represent the polysaccharide.) 1-4 represent individuals showing increasing amount of paraglycogen, about  $\times 200$ .

starvation was continued for a longer period. The other experiment gave exactly opposite results, the ciliates having but little reserve carbohydrate showing a decrease, while those in which the paraglycogen content was high, increased. In this instance also, striking results were obtained after four weeks, but a continued feeding of the same diet produced little further change. In both cases, therefore, the maximal changes had occurred within four weeks.

The two remaining experiments gave less significant results. With the high-protein diet, there was a slight increase in the carbohydrate reserve after four weeks, but at the end of eight weeks, the paraglycogen of the parasites dropped again to the amount originally present. On the high-fat diet, a slight decrease was followed by an increase in the percentage of reserve material present. It would appear that, on the whole, high-protein and high-fat diets have very little, if any, influence upon the polysaccharide storage.

TABLE 3.—*Influence of diet on polysaccharide storage in N. ovalis*

Showing percentage of each population which approached most nearly to each of the 4 chosen standards (see Fig. 1)

		Starvation	High-carbohydrate diet	High-protein diet	High-fat diet
		%	%	%	%
Beginning ..	Standard 1	12	10	16	16
	Standard 2	50	42	36	36
	Standard 3	32	40	34	34
	Standard 4	6	8	14	14
4 weeks ....	Standard 1	20	0	10	16
	Standard 2	76	30	30	64
	Standard 3	4	44	38	18
	Standard 4	0	26	22	2
8 weeks ....	Standard 1	16	2	4	12
	Standard 2	72	30	57	40
	Standard 3	8	38	34	34
	Standard 4	4	30	5	14

#### *Fat Reserves*

Lipoid reserves have been found in a large number of protozoa (von Brand, 1935). Consequently, a study of the fat reserves of the protozoa parasitic in the cockroach was included in this investigation. Generally, the staining methods employed failed to reveal fat in the form of visible droplets in any of the species being considered. However, after the hosts had been fed on a high-fat or a high-protein diet for six to eight weeks, droplets which stained a bright orange with Sudan III were sometimes present in *Endolimax blattae* and occasionally also in *N. ovalis*. Since the milk-powder used to provide a diet high in protein contained 26.5% of milk fat, it is not surprising that fat storage occasionally occurred in amoebae from hosts provided with this food.

#### *Size of N. ovalis*

Since many intestinal protozoa feed directly on the food of the host, it appeared of interest to determine whether or not size changes occur in the parasites when the diet of the host animal is varied. *N. ovalis* is best-suited for studies of this type because it is present more frequently and abundantly than some of the other species and is of larger size.

For each of the four previously mentioned diets, three test groups were used: (1) roaches just collected and not yet placed on a special diet, (2) animals fed on a

given diet (or starved) for four weeks, and (3) hosts placed on a particular diet (or starved) for eight weeks. Fifty specimens of *N. ovalis* from each test group were measured in the manner previously described. There was one exception since in starved animals the number of parasites present was so small that only twenty-five individuals per group were measured. The complete results of these studies are shown in Table 4.

TABLE 4.—Influence of diet on size (in  $\mu$ ) of *N. ovalis*

		Initial	4 weeks	8 weeks
Starvation .....	Length	85.2 $\pm$ 2.3	67.9 $\pm$ 2.9	54.5 $\pm$ 1.8
	Width	58.2 $\pm$ 1.8	48.7 $\pm$ 2.5	35.6 $\pm$ 1.5
High-carbohydrate diet ...	Length	73.5 $\pm$ 2.0	91.4 $\pm$ 3.7	90.0 $\pm$ 3.1
	Width	52.2 $\pm$ 1.8	67.5 $\pm$ 2.9	64.1 $\pm$ 2.3
High-fat diet .....	Length	79.3 $\pm$ 1.6	75.8 $\pm$ 1.4	67.1 $\pm$ 1.7
	Width	56.0 $\pm$ 1.5	54.3 $\pm$ 1.3	46.6 $\pm$ 1.2
High-protein diet .....	Length	79.3 $\pm$ 1.6	87.5 $\pm$ 2.5	80.9 $\pm$ 2.3
	Width	56.0 $\pm$ 1.5	62.7 $\pm$ 2.0	55.7 $\pm$ 1.7

Ciliates from starved roaches had decreased considerably in size at the end of four weeks and were still smaller after eight weeks. *Nyctotherus* from hosts fed on a high-carbohydrate diet were significantly larger after four weeks but no further increase had occurred after eight weeks. The high-protein diet also produced a certain increase in size to a maximum at the end of four weeks but not quite as much as with the high-carbohydrate diet. A high-fat diet produced very little change in the size of the ciliates for a relatively long period of time, but after eight weeks had elapsed, they were notably smaller than at the beginning.

#### Number of *N. ovalis* per Host

The experiments described above revealed that the percentage of roaches harboring *N. ovalis* remained essentially the same, regardless of the kind of food provided. However, it was thought worthwhile to determine whether or not the numbers of parasites per host could be influenced by the various diets.

After counts had been made for each series, the results were studied statistically to determine whether or not the increase or decrease in the numbers of *N. ovalis* was significant. The results of these studies are shown in Table 5.

TABLE 5.—Influence of diet on numbers of *N. ovalis* per host

	Initial	2 weeks	4 weeks
Starvation .....	203 $\pm$ 43.8	174 $\pm$ 28.6	107 $\pm$ 11.7
High-carbohydrate diet .....	203 $\pm$ 43.8	347 $\pm$ 68.5	539.6 $\pm$ 86.5
High-fat diet .....	203 $\pm$ 43.8	145.2 $\pm$ 23	139.7 $\pm$ 22.6
High-protein diet .....	203 $\pm$ 43.8	166.8 $\pm$ 30.5	159 $\pm$ 26.3

Several points of interest will be noted: (1) The high-carbohydrate diet was the only one which produced a significant increase in the numbers of ciliates present. The increase was most rapid during the first two weeks, after which time it was very slight. (2) The starved animals alone showed a significant decrease in numbers of *Nyctotherus*. During the first two weeks of starvation, the decrease was small but became statistically significant after four weeks had elapsed. (3) On each of the other diets, the numbers of *N. ovalis* became somewhat smaller, but in neither case was the decrease significant.



## DISCUSSION

The experiments reported in this paper were undertaken in order to determine the influence of starvation and of several different diets on certain intestinal protozoa of the cockroach. The starvation experiments resulted in the complete elimination of *Endolimax blattae* and *Lophomonas blattarum* while *Endamoeba blattae* showed a marked decrease in infection rate. There was little or no decrease in the number of host animals infected with *Nyctotherus ovalis* and *Lophomonas striata*. Varied results have been obtained by other investigators in similar experiments. Cleveland (1925b) found that termites starved for six days lost their infection with *Trichonympha* and if starved for eight days lost *Leidyopsis*, another type of intestinal flagellate. Hegner and Eskridge (1937a) concluded that trichomonads are not easily eliminated from rats by depriving the host of food. In the present study starvation of roaches resulted in a decrease in the size and numbers of *N. ovalis*, as well as in the amount of paraglycogen stored by this ciliate. These results were comparable with those obtained by Harding (1937b) when the ciliate, *Glaucoma*, was starved and by Mowry and Becker (1930) with the ciliates of ruminants.

Except for *N. ovalis*, the incidence of infection of the parasites studied remained highest when the roaches received food having a high-carbohydrate content. Sas-suchin (1931) reported similar results in his work on *Blatta orientalis*. Hegner and Eskridge (1937b) observed that a diet high in carbohydrates created a favorable environment for the amoebae in the caecum of the rat, and Mowry and Becker (1930) reported a doubling of the numbers of rumen infusoria with the addition of cornstarch to the ration of goats receiving only hay. A high-carbohydrate diet resulted in an increase in the size, amount of stored paraglycogen, and numbers per host of *N. ovalis*.

Saccuchin (1931) reported that the intestinal protozoa of the cockroach, *B. orientalis*, showed a decreased percentage of infection when a high-protein diet was supplied. On the other hand, Morris (1936) stated that meat or other high-protein food, such as milk powder, was of distinct advantage in maintaining good infections with *Endamoeba blattae* in all the common species of cockroaches. Hegner and Eskridge (1937b) observed that a diet high in protein but low in carbohydrates modified the caecal contents of rats so that amoebae were no longer able to live in this location. Hegner (1923) and Ratcliffe (1930) reported a reduction of caecal flagellates in rats on a high-protein diet, while Kessel (1929) and Kessel and Huang (1926) reported a decrease in the numbers of amoebae when an exclusive milk diet was given to rats, monkeys, and children. In the present work, when a high-protein diet was supplied to the roaches, the flagellates studied showed a decreased incidence as did those in the experiments described by other investigators. The amoebae of the cockroach were not eliminated by this type of diet, as were those of the rat. The protein diet produced no significant change in the numbers of *N. ovalis* per host and had little influence on the parasite's storage of polysaccharides. There was, however, an increase in the size of *N. ovalis* on this kind of diet.

Few papers have reported studies of the influence of high-fat diets, on the persistence of intestinal protozoa. In the present investigation this type of diet resulted in a decreased infection rate for flagellates and *Endamoeba blattae* but caused little change in the percentage of infection with *N. ovalis*. Providing the host with a high-fat diet did not significantly change the number of *N. ovalis* per host nor the

amount of its polysaccharide reserves, but the parasites became somewhat smaller in size. Fat storage was not frequently observed in any of the protozoan species considered but was induced in *Endolimax blattae* and occasionally in *N. ovalis* when a high-fat diet was supplied.

That modifying the diet of a host has a decided influence on its intestinal fauna has been clearly demonstrated by the results discussed in the foregoing pages. Several theories have been advanced as to the factors responsible for the changes observed. A high-carbohydrate diet in most cases is more favorable and a high-protein diet may be unfavorable. Hegner (1937) mentioned several possible factors that might be responsible for these results: (1) changes in hydrogen-ion concentrations, (2) the character of the bacteria resulting from different kinds of diets, and (3) the protozoa may actually require carbohydrate as a food element. Becker and Morehouse (1937) expressed the belief that reduced oöcyst production by the coccidian, *Eimeria miyairii*, when host rats were given skim-milk powder and certain other diets was due to the absence of a special nutrition-factor from the diet, rather than to a positive arrester of the parasite's development. The method of nutrition of the protozoa may have a limiting influence, especially on holozoic species. For example, Lund (1930) found that all of the intestinal protozoa of the termite, *Termopsis*, died very quickly when the host was given a diet of soluble materials. One of the reasons to which he attributed this was the fact that most of the protozoa of *Termopsis* are holozoic and if all of the food is in solution they are not able to ingest enough to keep alive.

Another possible explanation for the results obtained is that, because they live under anaerobic or partially anaerobic conditions, the intestinal parasites have a high-carbohydrate metabolism and consequently thrive on a diet rich in this kind of food. This view is supported by the fact that, in practically every case described, the intestinal forms were present more frequently and in larger numbers when the host was fed on a high-carbohydrate diet.

A condition that is unfavorable for one species of protozoön may not produce the same effect on other species. It is, therefore, likely that the factors responsible for the changes observed are not the same in every case but that under various circumstances many different ones may be concerned.

#### SUMMARY

1. The American cockroach, *Periplaneta americana*, was maintained under several conditions, including starvation, high-carbohydrate, high-fat, and high-protein diets.
2. The effect of these diets on the persistence and on the polysaccharide and fat reserves of five species of intestinal protozoa of the cockroach was observed.
3. Quantitative studies were also made to determine the influence of the diets on the polysaccharide storage, the size, and the numbers of *Nyctotherus ovalis*.
4. Host starvation lowered the incidence of infection for most of the species studied, while a high-carbohydrate diet maintained it at a relatively high level. However, the persistence of *N. ovalis* was apparently not influenced by the diet of the host.
5. Starvation of the host produced a decrease in the size and number of *N. ovalis*, as well as in the amount of paraglycogen stored by the species.

6. A high-carbohydrate diet resulted in an increase in the size, amount of stored paraglycogen, and numbers of *N. ovalis*.

7. Diets high in fat or protein, in general, exerted very little influence on the intestinal fauna of the cockroach.

8. Several factors which might be responsible for the results obtained are discussed.

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# THE EFFECT OF NICOTINE-BENTONITE AND OF CERTAIN PHYSICAL STATES UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS

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Phenothiazine has received greater study than any other anthelmintic used in veterinary medicine, although its usefulness in this field was discovered only a few years ago. This intensive study has led to many practical applications. However, the field of possible usefulness of phenothiazine is far from completely explored. Additional investigations are needed to determine the relative susceptibility to phenothiazine medication of the various *Trichostrongylus* spp. which occur in sheep, to assess more accurately the practical value of using somewhat larger doses of phenothiazine in sheep, to evaluate the efficacy of mixtures of certain drugs with phenothiazine, and to determine if phenothiazine-salt mixtures are useful for the control of parasites in animals other than sheep. For the most part, this article explores the efficacy of a mixture containing phenothiazine and nicotine-bentonite in chickens.

## HISTORICAL

The anthelmintic value of an especially prepared nicotine-bentonite compound (containing 5% nicotine expressed as alkaloid) for the removal of *Ascaridia galli* from chickens was first described by Taylor and Galloway (1936). Nicotine products, described earlier, which contained the alkaloid or its salts merely adsorbed on various earthy materials or ground drugs, have not proved quite so satisfactory for use in poultry, and may react quite differently when mixed with phenothiazine. Levine (1936) reported that nicotine-bentonite removed all of the *Ascaridia galli* present in 45 pullets at the time of treatment. Apparently no other tests with this particular nicotine-bentonite preparation have been published. However, data collected at this laboratory by Dr. Ray M. Batchelder show that 94 chickens eliminated 1164 *Ascaridia* (98 per cent of the total present) within 48 hours of treatment with tablets containing 25 grains of nicotine-bentonite. Furthermore, when Dr. Batchelder administered one-half of the above mentioned tablets to each of 46 chickens weighing 2 pounds or less, 828 *Ascaridia* or 91 per cent of the total present were removed by the treatment. Nicotine-bentonite is not effective for the removal of other parasites from the chicken.

Various investigators report that phenothiazine possesses only slight efficacy against *Ascaridia galli*, but that it does possess considerable efficacy for the removal of *Heterakis gallinae*. McCulloch and Nicholson (1940) reported that 0.05 to 0.5 grams of phenothiazine, per bird, would destroy 95 to 100 per cent of the *Heterakis* present. Certain of the *Heterakis* were found by these workers in the ceca at necropsy, but it was claimed that these worms were dead as proved by the absence of movement when they were stimulated with warm water. Apparently, this method of detecting the absence of life as employed by McCulloch and Nicholson was inadequate since later investigators found that all *Heterakis* retained following treatment

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were alive. However, the number of worms eliminated in the investigations of McCulloch and Nicholson, as well as the tests reported by Allen, Olivier and Peterson (1942), Roberts (1940), Knowles and Blount (1941), as well as Guthrie and Harwood (1942), indicate that phenothiazine in adequate doses is effective for removal of *Heterakis gallinae* from chickens.

#### MATERIALS AND METHODS

The drugs employed were obtained from the usual commercial sources. Since the conditions of dosing varied from experiment to experiment, these will be reported in detail for each test.

The chickens employed were purchased locally and came from three flocks. All birds were cockerels, and all birds from the same flock were of the same age. The birds from one flock were Brown Leghorns, from the second Anconas, and from the third a mixture of White Wyandottes and Silver Lace Wyandottes.

Because most of the birds employed were of a Mediterranean breed, and easily upset by handling, slight loss of weight during the course of an experiment is not significant. The safety of the drug must rest largely on the size of dose necessary to produce death, as shown below. Unfortunately, the Brown Leghorn cockerels contracted a contagious disease of the respiratory tract, which complicates somewhat the interpretation of results. Since birds obviously suffering severely from respiratory disease were not used, this factor was reduced to a minimum, but in one or two instances, noted herein, the effects of the extraneous disease may have been important.

Only *Ascaridia* and *Heterakis* were studied carefully in these experiments, but it was noted that these birds were also infected with *Capillaria spp.*, *Davainea proglottina*, *Hymenolepis carioca*, *Raillietina cesticillus*, and *Choanotaenia infundibulum*. Before treatment, birds were placed in individual cages, in which they remained until they were killed. Every 24 hours intervening between treatment and death, the feces eliminated by each bird were collected, washed in screens, and searched for worms. Two to four days after treatment, the birds were killed, and the intestinal tracts searched for worms. The efficacy of the treatment was determined in the usual manner. The objection that dead worms may be destroyed by digestion, though valid with some species of parasites, is easily refuted in case of *Heterakis gallinae* and *Ascaridia galli* by direct observation of the worms. Also, in a portion of these tests untreated controls showed that only an insignificant number of the parasites are eliminated in the absence of treatment.

This method of conducting anthelmintic research, which is known as Hall's critical test, has been criticized of late by a few investigators because certain statistical methods have not been applied to results obtained by it. However, Hall's critical test enables one to determine with precision the size of a population exposed to a given amount of the drug, and the effect of the drug on that population. Therefore, one basis of variation inherent to all other methods of studying anthelmintics is removed.

Nevertheless uncontrolled variables exist in the experiments which are reported in this article, namely, the number of worms in each bird and the relative efficacies of the same treatment in each of several birds. Statistical control of these variables may be desirable, but it is difficult to achieve as acknowledged by the authors of Joint Publication No. 4 (1943: apparently B. G. Peters and R. A. Fisher). On the other

hand, the extensive experience of the Zoological Division, United States Bureau of Animal Industry, which has introduced many useful anthelmintics, indicates that the results of the critical test may be assessed with a very practical degree of precision by direct inspection. On this basis, the recent attempts to discredit reports of anthelmintic research which were conducted by methods of established usefulness are deprecated. The writers find that most reports dealing with phenothiazine as an anthelmintic are useful, interpretable and reasonably consistent. The inconsistent results reported by Peters, Leiper and Clapham (1941), but refuted in Joint Publication No. 4 (1943), were obtained by ignoring certain well-known characteristics of the helminths studied, namely, the rates of acquisition and development of these parasites. Consequently, the writers believe that Hall's critical test is a method of established value when suitably employed. Because helminths vary greatly in structure, habits and habitats, other methods, which may involve analysis of variables, are likewise valuable, but need not be discussed at this time.

#### EXPERIMENTAL RESULTS

As the first three experiments were orientation tests, a summary of the results obtained, rather than the usual presentation of individual records will be reported.

*Experiment 1.* In this test 0.25 grams of phenothiazine and 0.56 grams of nicotine-bentonite were placed in each of 11 hard gelatin capsules, and given to each of 11 Brown Leghorn cockerels weighing from 785 to 1,000 grams each. The birds were killed 48 hours after treatment. Of the 33 *Ascaridia* present, 29 (88 per cent) were removed by the treatment, and of the 190 *Heterakis* present, 131 (68.9 per cent) were removed. All the *Ascaridia* were removed from 9 chickens, and all the *Heterakis* from 3 chickens. From 4 chickens the treatment removed less than 70 per cent of the *Heterakis*, a figure which is often used arbitrarily as the minimum for satisfactory efficacy.

These birds were killed only two days after treatment, but many *Heterakis* were eliminated during the second day after treatment, for instance, 9 out of a total of 12 *Heterakis* present in one bird were eliminated on the second day. This suggests that more of these parasites may have been destroyed and would have been eliminated had the birds been retained longer. In all subsequent experiments the birds were held at least three days before necropsy.

*Experiment 2.* For this test tablets weighing  $1.242 \pm 0.034$  grams were prepared from a formula containing phenothiazine, 1 part, and nicotine-bentonite, 3 parts. On August 20, 1 tablet was given to each of 7 Brown Leghorn cockerels weighing more than 950 grams each and  $\frac{1}{2}$  of a tablet to 5 additional cockerels weighing less than 950 grams. These birds eliminated 90.7 per cent of 150 *Ascaridia* and 73.5 per cent of 1332 *Heterakis* present at the time of treatment. All the *Ascaridia* were removed from only 4 of the 9 chickens harboring these parasites, and all the *Heterakis* from only 2 of 12 chickens. Four of the birds eliminated less than 70 per cent of the *Heterakis* present.

*Experiment 3.* For this test tablets weighing  $1.33 \pm 0.029$  grams each were prepared from a formula containing phenothiazine, 1 part, and nicotine-bentonite, 2 parts. On August 8, 1 tablet was given to each of 7 birds weighing more than 950 grams and  $\frac{1}{2}$  of a tablet to each of 5 birds weighing less than 950 grams. These birds eliminated 94.6 per cent of 111 *Ascaridia* and 93.5 per cent of 650 *Heterakis* present at the time of treatment. All *Ascaridia* present were removed from 7 birds

and all *Heterakis* from 4 birds. One chicken eliminated less than 70 per cent of the *Heterakis* present. Since the formula containing the greater quantity of phenothiazine appeared to be the more effective it was employed in several additional experiments.

*Experiment 4.* The phenothiazine nicotine-bentonite tablets used in experiment three were scored in the center in order that they might be broken in two approximately equal parts for treating small birds. Each  $\frac{1}{2}$  tablet was then weighed and given to a cockerel weighing less than 950 grams. These weights are recorded with other pertinent data in Table 1. The treatment destroyed 84.0 per cent of 94 *Ascaridia* and 73.2 per cent of 1093 *Heterakis*.

TABLE 1.—Efficacy of one-half of a tablet\* containing 1 part of phenothiazine and two parts of nicotine-bentonite for the removal of *Heterakis* and *Ascaridia* from Brown Leghorn cockerels

Band No.	Weight at time of treatment†	Dose	Number of worms removed		Number of worms found at necropsy		Per cent efficacy	
			<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>
	Grams	Grams						
2675	835	0.700	2	5	5	14	28.6	26.3
2695	830	0.670	5	23	0	5	100.0	82.1
2278	935	0.688	1	76	0	0	100.0	100.0
2699	880	0.710	12	49	0	19	100.0	72.1
2697	870	0.704	1	22	1	170	50.0	11.5
2696	915	0.691	1	45	1	43	50.0	51.1
2244	855	0.688	25	48	1	0	96.2	100.0
2682	895	0.660	9	82	1	3	90.0	96.5
2276	870	0.700	16	23	6	4	72.7	85.2
2698	940	0.673	2	28	0	28	100.0	50.0
2648	915	0.680	3	180	0	0	100.0	100.0
2649	740	0.692	2	146	0	80‡	100.0	96.9
Total	...	....	79	727	15	366‡	....	....

\* For this experiment six tablets were selected at random and broken in two pieces in the fingers. The halves were then weighed carefully and one-half tablet administered to each of the 12 birds shown in the table.

† These birds were treated August 31, 1942, and were necropsied 3 days later.

‡ Bird No. 2649 developed very severe symptoms of respiratory embarrassment the day after treatment and ate very little throughout the course of this experiment. At necropsy 73 of the 80 *Heterakis* found were discolored and partially digested. The remaining 7, though normal in appearance did not move. The partially digested worms were believed to have been destroyed by the drug and are considered as "worms removed" in calculating the per cent of efficacy. All worms normal in appearance were considered to be alive.

*Experiment 5.* This experiment was designed to study both the efficacy of the tablet described in Experiment 3, and the comparative efficacy of the individual ingredients used in the manufacture of the tablet. Four birds were selected at random. To the first bird, one tablet was given; to the second, 0.44 gram of phenothiazine (a quantity equivalent to the amount used in the manufacture of the tablet); to the third, 0.88 gram of nicotine-bentonite; and to the fourth, an empty gelatin capsule. Four groups of birds were studied at one time, as more could not be handled simultaneously. However, this experiment was composed of 3 parts, conducted in 3 successive weeks, so that a total of 12 birds received each treatment. Detailed results of the test are presented in Table 2.

The birds receiving the tablet eliminated 96.2 per cent of 131 *Ascaridia* and 83.7 per cent of 1012 *Heterakis*. Since the birds receiving no treatment eliminated only 3.8 per cent of 131 *Ascaridia* and 0.9 per cent of 1142 *Heterakis*, the efficacy of the tablet is apparent. Nicotine-bentonite alone removed 86.8 per cent of 159 *Ascaridia* and 10.1 per cent of 1246 *Heterakis*. Apparently nicotine-bentonite at the dose rate employed possesses very slight efficacy against *Heterakis*, and considerable efficacy against *Ascaridia*. Phenothiazine alone removed 43 per cent of 100 *Ascaridia* and

89.9 per cent of 675 *Heterakis*. These results are in keeping with earlier investigations which indicate that phenothiazine is effective for the removal of *Heterakis* but only slightly effective for the removal of *Ascaridia*.

TABLE 2.—Comparative elimination of *Ascaridia* and *Heterakis* from Broken Leghorn cockerels which received four different treatments

Band No.	Weight at time of treatment* (grams)	Date of treatment (1942)	Number of worms eliminated		Number of worms found at necropsy		Per cent efficacy	
			<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>
Birds dosed with tablets†								
2279	1200	Sept. 8	1	26	0	7	100.0	78.8
2283	1360	Sept. 8	10	261	2	2	83.3	99.2
2287	1330	Sept. 8	9	0	0	0	100.0	....
2293	1220	Sept. 8	12	10	0	0	100.0	100.0
2296	1065	Sept. 15	1	28	0	15	100.0	65.1
2300	1000	Sept. 15	34	26	0	2	100.0	92.9
2956	1170	Sept. 15	31	252	1	16	96.9	94.0
2960	1210	Sept. 15	23	66	2	68	92.0	49.3
2964	1260	Sept. 21	0	37	0	48	....	43.5
2968	1265	Sept. 21	1	63	0	7	100.0	90.0
2972	1100	Sept. 21	1	13	0	0	100.0	100.0
2977	1185	Sept. 21	3	65	0	0	100.0	100.0
Total	....	....	126	847	5	165	....	....
Birds dosed with phenothiazine in hard gelatin capsules†								
2282	1365	Sept. 8	0	35	0	0	....	100.0
2286	1290	Sept. 8	3	1	13	0	18.8	100.0
2290	1125	Sept. 8	4	38	4	0	50.0	100.0
2292	1220	Sept. 8	0	28	0	0	....	100.0
2297	1180	Sept. 15	18	79	20	0	47.4	100.0
2953	1400	Sept. 15	2	22	0	21	100.0	51.2
2957	1135	Sept. 15	2	10	5	14	28.6	41.7
2961	1115	Sept. 15	1	69	0	0	100.0	100.0
2965	1160	Sept. 21	5	88	6	3	45.5	96.7
2969	1150	Sept. 21	2	27	1	29	66.7	48.2
2973	1065	Sept. 21	3	116	0	0	100.0	100.0
2978	1285	Sept. 21	3	94	8	1	27.3	98.9
Total	....	....	43	607	57	68	....	....
Birds dosed with nicotine-bentonite in hard gelatin capsules†								
2285	1200	Sept. 8	22	52	5	171	81.5	23.3
2289	1230	Sept. 8	0	2	0	31	....	8.8
2281	1165	Sept. 8	6	2	0	114	100.0	1.7
2294	1440	Sept. 8	7	23	15	140	31.8	2.1
2298	1330	Sept. 15	17	25	0	109	100.0	18.7
2954	1130	Sept. 15	0	4	0	49	....	7.5
2958	1070	Sept. 15	5	3	0	75	100.0	3.8
2962	1175	Sept. 15	3	12	0	15	100.0	44.4
2966	1053	Sept. 21	46	0	0	133	100.0	0.0
2970	1110	Sept. 21	13	5	0	159	100.0	3.0
2974	1090	Sept. 21	0	0	0	42	....	0.0
2979	1050	Sept. 21	19	17	1	82	95.0	17.2
Total	....	....	138	126	21	1120	....	....
Birds dosed with an empty hard gelatin capsule								
2288	1090	Sept. 8	2	1	38	150	5.0	0.7
2280	1200	Sept. 8	0	0	14	189	0.0	0.0
2284	1185	Sept. 8	1	0	8	84	11.1	0.0
2295	1275	Sept. 8	0	2	3	72	0.0	2.7
2299	1085	Sept. 15	0	0	9	51	0.0	0.0
2955	1225	Sept. 15	0	0	9	24	0.0	0.0
2959	1210	Sept. 15	2	3	28	234	6.7	1.3
2963	1295	Sept. 15	0	0	0	213	....	0.0
2967	1025	Sept. 21	0	4	7	33	0.0	10.8
2971	1185	Sept. 21	0	0	0	30	....	0.0
2976	990	Sept. 21	0	0	2	37	0.0	0.0
2980	1340	Sept. 21	0	0	8	15	0.0	0.0
Total	....	....	5	10	126	1132	....	....

\* All birds were necropsied 3 days after treatment.

† The tablets employed weighed 1.33 grams, and were made of phenothiazine, 33 per cent; nicotine-bentonite (containing 5 per cent nicotine), 66 per cent; and lubricant 1 per cent. The dose of phenothiazine employed was 0.44 gram, and of nicotine-bentonite, 0.88 gram.

Possibly phenothiazine and nicotine-bentonite possess an additive action against *Ascaridia* since the birds treated with the tablet eliminated 96.2 per cent of the



worms present at the time of treatment, while the birds receiving nicotine-bentonite eliminated only 86.9 per cent of the total present. However, the evidence for additive action is far short of proof since 3 of the birds receiving each treatment harbored *Ascaridia* at necropsy. In these tests the tablet removed 6 per cent fewer *Heterakis* than an equivalent amount of phenothiazine. Furthermore, 7 of the birds receiving phenothiazine alone harbored no *Heterakis* at necropsy, while only 3 of the birds receiving the tablet were negative for the cecal worm at necropsy. This suggests the possibility of interference in therapeutic activity between nicotine-bentonite and phenothiazine, a suggestion which is supported by Mohler (1941). Additional evidence on this point will be presented in experiment 8.

*Experiment 6.* Since the dosage of anthelmintics is ordinarily regulated by weight of the host, a group of large Wyandotte cockerels weighing from 2.5 to 3 kilograms were obtained, and each was given one of the tablets described under experiment 3. Details of the experiment are presented in Table 3. The treatment removed 93.7 per cent of 202 *Heterakis* and the 2 *Ascaridia* which were present.

TABLE 3.—Efficacy of a tablet\* containing phenothiazine and nicotine-bentonite for the removal of *Heterakis* from Wyandotte cockerels†

Band No.	Weight at time of treatment, November 10	Number of <i>Heterakis</i> removed	Number of <i>Heterakis</i> found at necropsy	Per cent efficacy for <i>Heterakis</i>
	Grams			
2891	2925	8	0	100.0
2892	2835	0	0	....
2882	2475	13	0	100.0
2884	2025	25	0	100.0
2888	2835	8	10	44.4
2889	3015	35	0	100.0
2890	2880	14	2	87.5
2885	3015	47	0	100.0
2886	2565	36	1	97.3
2887	2250	2	1	66.7
Total	....	188	14	....

\*The tablet used contained 1 part of phenothiazine and 2 parts of nicotine-bentonite, plus 1 per cent lubricant. It weighed  $1.33 \pm 0.029$  grams.

†The birds used in this test harbored only 2 *Ascaridia*, one each in birds 2892 and 2890. Both worms were removed by the treatment.

*Experiment 7.* Phenothiazine sufficiently fine to pass through a screen of 12 meshes to the inch, but so coarse that it was retained by a screen of 16 meshes to the inch was obtained. This material was made into tablets similar to those described in experiment 3. One tablet was given to each of 10 Ancona cockerels. The results of this experiment are presented in Table 4. As the tablet removed 95.2 per cent of 62 *Ascaridia* and 92.3 per cent of 889 *Heterakis*, there is little indication that the coarseness of the phenothiazine in any way affected the efficacy of the tablet.

*Experiment 8.* As both phenothiazine and nicotine-bentonite are frequently administered to chickens in mixtures of feed, an effort was made to study the mixture of the two compounds when administered in this manner. For this purpose 3 grams of phenothiazine and 4.5 grams of nicotine-bentonite were mixed with 115 grams of an all-mash feed and placed before 6 cockerels confined in one cage. For purposes of comparison, a similar mixture containing phenothiazine alone was placed before 6 cockerels of the same age and breed. Because chickens cannot readily obtain all of the feed from a metal trough, twice the above described dose was placed before the birds and the amount consumed was determined by weighing back the residue when about  $\frac{1}{2}$  the total had been consumed. Thus the dose actually administered

was greater than originally intended. As these birds were treated in groups, individual records are not available for tabulation. This experiment consists of two tests; in the first test Brown Leghorns were used; in the second, Anconas. The six Brown Leghorn cockerels dosed on October 5 with phenothiazine and nicotine-bentonite actually consumed 128 grams of medicated feed or 5.5 grams more than the intended dose. Fifty-two *Ascaridia* and 226 *Heterakis* were eliminated following treatment. As 4 *Ascaridia* and 15 *Heterakis* were found at necropsy, the percentage of efficacy was 92.9 for *Ascaridia* and 93.8 for *Heterakis*. The 6 cockerels dosed simultaneously with phenothiazine alone consumed 120 grams of medicated mash, or 2 grams more than the intended dose. They eliminated 66.7 per cent of 45 *Ascaridia*, and 94 per cent of 200 *Heterakis* present at the time of treatment.

On November 28, 6 Ancona cockerels were given 9.4 grams of phenothiazine and 14.9 grams of nicotine-bentonite in 238 grams of feed. This dose was somewhat

TABLE 4.—Efficacy of a tablet\* containing coarse phenothiazine and nicotine-bentonite for the removal of *Ascaridia* and *Heterakis* from Ancona cockerels†

Band No.	Weight at time of treatment	Number of worms removed		Number found at necropsy		Per cent efficacy	
		<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>
	Grams						
2785	1815	1	102	0	0	100.0	100.0
2786	2045	0	20	0	2	100.0	90.9
2787	1810	0	13	0	55	100.0	19.1
2788	1665	14	51	0	8	100.0	86.4
2789	1865	2	20	0	2	100.0	90.9
2790	1725	1	21	0	0	100.0	100.0
2791	1975	0	20	0	0	100.0	100.0
2792	1655	19	206	3	1	86.4	99.5
2793	1545	18	265	0	0	100.0	100.0
2794	1940	4	103	0	0	100.0	100.0
Total		59	821	3	68		

\* This tablet weighing 1.34 grams contained 1 part of coarse phenothiazine (16 mesh) and 2 parts of nicotine-bentonite to which 1 per cent of a lubricant was added.

† The cockerels were dosed December 18, 1942, and necropsied 3 days later.

excessive. The birds eliminated following treatment all of 57 *Ascaridia*, but only 67.2 per cent of 765 *Heterakis*. Of the total of 251 *Heterakis* found at necropsy 241 were found in one bird which may not have consumed its full share of the medicated mash. The 6 Ancona cockerels dosed simultaneously with phenothiazine alone received 10.4 grams of phenothiazine from 283 grams of mash. They eliminated 88 per cent of 25 *Ascaridia* and 76.1 per cent of 477 *Heterakis*. As 89 of the 114 *Heterakis* recovered at necropsy were in one bird, it is possible that this bird did not eat a sufficient quantity of the mash to obtain a full dose.

Because of the inadequate control of dosage for each bird in these tests involving mass feeding, it is doubtful whether a valid comparison between the efficacy of phenothiazine and of a mixture of phenothiazine with nicotine-bentonite was obtained. However, it may be noted that in both tests phenothiazine alone was at least as effective as the mixture.

*Experiment 9.* To test the toxicity of phenothiazine mixed with nicotine-bentonite, each of 23 Brown Leghorn cockerels, which seemed to be healthy at the time, were given on August 31 several tablets similar to those described in experiment 3. The birds were confined to individual cages for one week following treatment, and then released in a pen 15 feet by 20 feet. The test was terminated 3 weeks after treatment. Five tablets were administered to each of 6 cockerels weigh-

ing from 970 to 1200 grams at the time of treatment. As all 6 birds gained weight normally, a dose of this size seemed to be relatively harmless. Ten tablets were administered to each of 12 cockerels weighing from 1025 to 1400 grams at the time of treatment. Five days later all but one of the twelve birds had gained weight, indicating that little or no injury had resulted from this dose. However, during the second week after treatment, shortly after the release of these birds in a common pen, one cockerel which had received 10 tablets was found dead. The appearance at necropsy suggested that injuries received while fighting were the cause of death. Twenty tablets were given to each of four cockerels, weighing from 1020 to 1570 grams at the time of treatment. Five days after treatment 3 of the 4 birds weighed

TABLE 5.—Efficacy of 0.5 grams of "wetable" phenothiazine compared with 0.5 grams of regular commercial phenothiazine for the removal of *Ascaridia* and *Heterakis* from Ancona cockerels

Band No.	Date of treatment	Weight at time of treatment (grams)	Worms removed by treatment		Worms found at necropsy		Per cent removed	
			<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>	<i>Ascaridia</i>	<i>Heterakis</i>
Birds dosed with "wetable" phenothiazine								
2781	(1942) Dec. 8	1975	6	39	0	0	100.0	100.0
2782	Dec. 8	1855	1	114	0	0	100.0	100.0
2783	Dec. 8	1640	1	189	0	0	100.0	100.0
2784	Dec. 8	1980	0	108	0	0	....	100.0
3586	(1943) Jan. 4	1745	24	5	0	0	100.0	100.0
3587	Jan. 4	1735	8	191	5	34	61.5	84.9
3588	Jan. 4	1860	1	72	8	0	11.1	100.0
3589	Jan. 4	1905	1	137	3	0	25.0	100.0
3590	Jan. 4	1845	51	453	17	0	75.0	100.0
Total	....	....	93	1308	33	34	....	....
Birds dosed with regular phenothiazine								
2777	(1942) Dec. 8	2240	0	1	0	0	....	100.0
2778	Dec. 8	1730	0	196	8	1	0.0	99.5
2779	Dec. 8	1950	0	15	0	0	....	100.0
2780	Dec. 8	1800	0	21	2	0	0.0	100.0
3581	(1943) Jan. 4	1665	2	26	0	0	100.0	100.0
3582	Jan. 4	1785	2	58	4	3	33.3	95.1
3583	Jan. 4	1870	2	113	0	0	100.0	100.0
3584	Jan. 4	2000	0	6	0	42	....	12.5
3585	Jan. 4	1675	22	30	0	0	100.0	100.0
Total	....	....	28	466	14	46	....	....

less than on the day of treatment. However, recovery was prompt since all birds gained in weight between the 5th and 7th days after treatment. One cockerel weighing 1190 grams was given 40 tablets, approximately all the crop would hold. As this dose contains 1.76 grams of nicotine alkaloid, it seemed likely that it would prove fatal. However, this bird which weighed 1190 grams August 31, weighed 1010 grams September 5, 1085 grams September 7, 1235 grams September 14, and 1315 grams September 21. Apparently, chickens are able to withstand excessively large doses of nicotine in the form used for manufacture of these tablets.

*Experiment 10.* A number of substances are used to make suspensions of phenothiazine that may be administered as a drench to sheep and other animals. Some of these wetting and dispersing agents may possibly affect the therapeutic activity of the phenothiazine. Therefore, an experiment was designed to compare the effectiveness of a "wetable" phenothiazine (containing an admixture of 1.3 per cent of algin and gum karaya) with the regular, commercial product. In this

test one-half of a group of Ancona cockerels were treated with 0.5 grams of "wetttable" phenothiazine and the remaining half with 0.5 grams of regular phenothiazine. Results are detailed in Table 5. The "wetttable" phenothiazine removed 73.8 per cent of 126 *Ascaridia* and 97.5 per cent of 1342 *Heterakis* present at the time of treatment. The regular phenothiazine removed 66.7 per cent of 42 *Ascaridia* and 91 per cent of 512 *Heterakis*. Apparently the wetttable phenothiazine was at least as effective as the regular phenothiazine since it removed a greater percentage both of *Ascaridia* and of *Heterakis*.

#### DISCUSSION

In these tests, mixtures of phenothiazine with nicotine-bentonite proved effective for the removal of *Ascaridia* and *Heterakis* from chickens, and the mixture seems to be reasonably safe for use since a dose of crop capacity did not cause the death of a cockerel. However, this conclusion cannot be extended to other preparations containing nicotine because many nicotine-preparations are far more toxic than the special nicotine-bentonite used in these tests.

The possible effect of the state of particulation of phenothiazine upon its anthelmintic activity has been discussed occasionally because the drug is so insoluble that large particles which present relatively less surface area might prove ineffective. Guthrie and Harwood (1942) found that micronized phenothiazine was no more effective than phenothiazine of regular grind for the removal of *Ascaridia* and *Heterakis* from chickens. A test reported in this paper (experiment 7) suggests that coarsely ground phenothiazine is as effective against *Heterakis* as that which is ground in the usual manner. These results suggest that within reasonable limits the state of particulation of phenothiazine is not important in the anthelmintic action of the drug when used in chickens.

In the course of these experiments, and of similar experiments reported by Guthrie and Harwood (1942), as well as Harwood and Guthrie (1944), several doses of phenothiazine were used; doses of 0.15 gram of phenothiazine per bird removed 57.5 per cent of 1666 *Heterakis* present in 20 Brown Leghorn cockerels (Guthrie and Harwood, 1942); doses of 0.2 gram per bird removed 60.8 per cent of 926 *Heterakis* present in 6 Ancona cockerels (Harwood and Guthrie, 1944); doses of 0.44 gram per bird removed 89.9 per cent of 675 *Heterakis* present in 12 Brown Leghorn cockerels; and doses of 0.5 gram per bird removed 95.6 per cent of 1853 *Heterakis* from 18 Ancona cockerels. These results indicate that the efficacy of phenothiazine for the removal of *Heterakis* is correlated with the dose, and that a dose of 0.5 gram per bird approaches the optimum. Doses higher than 0.5 gram per bird should be used cautiously since Nicholson and McCulloch (1942) found that repeated doses of 1 gram of phenothiazine per bird affects laying hens deleteriously.

Although nicotine-bentonite possesses slight efficacy against *Heterakis*, phenothiazine alone was more effective for the removal of this parasite than mixtures of phenothiazine with nicotine-bentonite in the three tests described under experiment 5, as well as in the two tests described under experiment 8. In some instances the difference was very slight. Six and two-tenths, 4.9, 14.4, 0.2 and 8.9 per cent more *Heterakis* were removed from birds treated with phenothiazine than from similar birds treated simultaneously with mixtures of phenothiazine and nicotine-bentonite.



The probability that the mean difference (6.92 per cent) was due to chance is less than 5 per cent. Therefore, it is likely that bentonite, which is used extensively for making suspensions of phenothiazine, depresses slightly the anthelmintic activity of phenothiazine. However, in the case of *Heterakis* the slightly lower efficacy of the mixture is not important.

Considering the differences in technique employed, our results are in very good agreement with the results obtained by other American workers (Allen, Olivier and Peterson, 1942; McCulloch and Nicholson, 1940), but foreign workers (Roberts, 1940; Knowles and Blount, 1941) have generally obtained poorer results. Possibly these differences in results may be due to differences in the techniques employed. However, differences in the character of the phenothiazine employed may possibly explain the different degrees of efficacy obtained since Roberts, as well as Knowles and Blount, used phenothiazine containing 5 per cent of a phenol. Apparently, the effect of admixtures of phenols upon the anthelmintic efficacy of phenothiazine has not been studied.

#### SUMMARY

1. To investigate the possible usefulness of a mixture containing phenothiazine and nicotine-bentonite over 100 naturally infected cockerels of three breeds were employed. A tablet weighing  $1.33 \pm 0.29$  grams proved effective for the removal of both *Ascaridia* and *Heterakis* from chickens. Furthermore, a dose of 40 tablets did not prove fatal to 1 cockerel weighing 1190 grams, although, this dose contained 1.75 grams of nicotine.

2. The use of coarse ground phenothiazine, or of micronized phenothiazine did not affect appreciably the value of the compound for the removal of *Heterakis* from chickens.

3. A "wetable" grade of phenothiazine proved as effective as ordinary commercial phenothiazine for the removal of *Ascaridia* and *Heterakis* from the chickens used in these tests.

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### THREE MONOGENETIC TREMATODES FROM MARINE FISHES\*

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This paper is a description of two new monogenetic trematodes found on the gills of marine fishes in the region of Tortugas, Florida, and a redescription of another found both at Tortugas and Bermuda. The trematodes from Tortugas were collected in 1930-1932 by Dr. H. W. Manter while at the Tortugas Biological Laboratory of the Carnegie Institution of Washington, D. C. The Bermuda specimens were collected by Dr. F. D. Barker some years ago and were made available through the kindness of the General Biological Supply House.

Specimens were collected by scraping the gills of fishes in dishes containing sea water. They were killed in formal-alcohol-acetic solution under slight pressure of a cover glass, preserved in 70% alcohol, stained with Delafield's hematoxylin, and mounted in balsam.

The writer wishes to express thanks to Dr. H. W. Manter, University of Nebraska, under whose direction these studies were made, and to Dr. Emmett W. Price for certain suggestions.

Family HEXOSTOMATIDAE Price, 1936

*Hexostoma macracanthum* n. sp.

(Figs. 1-5)

*Description:* Body 3.57 to 4.25 mm long by 0.77 mm in greatest width, anterior end tapering and pointed but broadening out greatly near level of anterior end of ovary (at anterior fourth of body), followed immediately by a slight constriction and again broadening out slightly toward the posterior region. Posterior portion of body with four pairs of suckers, the anteriormost pair measuring 0.220 mm long by 0.318 mm wide, while the smaller, posteriormost pair measures 0.140 mm long by 0.220 mm wide. Each sucker with three longitudinal chitinous pieces, a large X-shaped piece in the middle and two broad, flat pieces imbedded in the lateral walls. Posterior end of body with two pairs of hooks, a large pair measuring 0.108 to 0.120 mm long by 0.010 mm in greatest width, and a small pair measuring 0.024 mm long. Mouth subterminal. Oral suckers elliptical, 0.034 to 0.047 mm long by 0.027 to 0.029 mm wide. Pharynx ovoid, 0.060 to 0.068 mm long by 0.030 to 0.036 mm wide. Esophagus with several lateral branches anterior to main bifurcation. Ceca much branched, uniting posteriorly by means of numerous, fine, lateral branches. Genital pore ventral, median. Genital atrium conical, 0.059 to 0.068 mm long by 0.051 mm in greatest width. Testes irregular in shape, post-ovarian, arranged more or less in two rows, 34 to 36 in number. Ovary median, near anterior third of body, a somewhat inverted U-shaped organ. Seminal receptacle near posterior right end of ovary, large, 0.24 to 0.28 mm long by 0.068 to 0.084 mm wide. Vaginal opening dorsal, immediately posterior to genital atrium, provided with a pair of cuticular, denticulated bodies, each measuring 0.060 to 0.066 mm long by 0.017 to 0.021 mm wide. Vitellaria extend from level of cuticular bodies to anterior end of posterior third of body. Eggs 0.103 to 0.187 mm long by 0.044 to 0.051 mm wide; anterior filament 0.028 to 0.040 mm long; posterior filament 0.119 to 0.161 mm long. Excretory pores situated anterior to genital pore on dorso-lateral margins of body.

This description is based on two specimens.

*Host:* *Euthymus alleteratus* (Rafinesque), little tunny.

*Location:* Gills.

*Locality:* Tortugas, Florida.

*Type:* U. S. Nat. Mus. Helm. Coll. No. 36890.

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\* Studies from the Zoological Laboratory, University of Nebraska, No. 220.

*Comparisons.*—*Hexostoma macracanthum* is most like *H. euthynni* Meserve, 1938 in body length and in the size of the anteriormost pair of posterior suckers. It differs from *H. euthynni* mainly in the smaller size of the posteriormost pair of suckers, the structure of the chitinous pieces of the posterior suckers, the number of testes, and in the lengths of the egg filaments (especially that of the anterior filament). Six other species of *Hexostoma* have been named. In proportion to body length, *H. macracanthum* possesses the largest posterior hooks.

Family DICLIDOPHORIDAE Fuhrmann, 1928

Subfamily CYCLOCOTYLINAE Price, 1943

*Cyclocotyla hysteroncha* n. sp.

(Figs. 6-8)

*Description:* Body (including haptor) 1.471 to 2.210 mm long by 0.328 to 0.460 mm wide, anterior end tapering and pointed. Posterior haptor approximately one-fourth total body length, with four pairs of pedicled suckers of approximately equal size, 0.098 to 0.101 mm in diameter. Pedicels of equal length, 0.109 to 0.124 mm. Languette very minute, with one pair of hooks approximately 0.028 mm long (Fig. 7). Skeleton of suckers composed of 9 large chitinous pieces (3 paired and 3 unpaired) and 5 or 6 pairs of small, curved lateral bars (Fig. 8). Musculature of sucker more or less divided into four saucer-shaped regions of which one is well developed while either two or three of the others may be greatly reduced (Fig. 8). As a result the musculature of the sucker is often asymmetrical. Mouth subterminal; buccal cavity with a pair of elliptical suckers, 0.028 to 0.032 mm long by 0.024 to 0.028 mm wide. Pharynx relatively large, 0.068 to 0.076 mm long by 0.044 to 0.052 mm wide. Intestinal ceca uniting at level of anterior edge of posterior haptor, with branches extending into posterior haptor and bases of the pedicels. Genital aperture median, immediately posterior to bifurcation of esophagus. Cirrus armed with 6 to 8 bifid hooks, 0.008 to 0.012 mm long (curvature not considered). Testes 6 to 7, post-ovarian, intercecal, smooth, resembling vitellaria in size and shape. Ovary median, near middle of body. Seminal receptacle large, pre-ovarian. Vitellaria filling most of body from level of genital aperture and extending into the haptor. Vagina lacking. No eggs observed. Excretory system not observed. Scattered pigment spots occur near surface of several specimens.

This description is based on five specimens.

*Hosts:* *Bathystoma striatum* (Linn.) (type host), *Brachygenys chrysargyreus* (Günther) and *Haemulon flavolineatum* (Desmarest).

*Location:* Gills.

*Locality:* Tortugas, Florida.

*Type:* U. S. Nat. Mus. Helm. Coll. No. 36891.

*Comparisons.*—The most distinctive features of *Cyclocotyla hysteroncha* are the small number of testes; the relatively large pharynx; and the presence of hooks on the languette.

*C. hysteroncha* seems to be most similar to *C. elongata* (Goto, 1894), *C. chrysophryi* (Beneden and Hesse, 1863), and *C. labracis* (Cerfontaine, 1895), but differs from all three in body size, in number of testes and cirrus hooks, and in the presence of one pair of hooks on the languette.

Price (1943) considered *Choricotyle* Beneden and Hesse, 1863 a synonym of *Cyclocotyla* Otto, 1823. The subfamily *Cyclocotylinae* Price, 1943 is distinguished from the *Diclidophorinae* Cerfontaine, 1895 by the presence of sucker-like musculature in the claspers. Llewellyn's recent (1941) review of the family Diclidophoridae places most species in the genus *Choricotyle*. Frayne (1943) redescribed "*Diclidophora cynoscioni* (MacCallum, 1917)" which he placed in the genus *Choricotyle*. Price (1943) placed this species in his new genus *Neoheterobothrium*, characterized by a long, slender isthmus between the haptor and the body, and by the vitellaria not entering into the haptor. The known species both possess a languette with two pairs of hooks. Frayne described the vitellaria of his *C. cynoscioni* as

entering the haptor. A specimen of this species collected from the type host at Beaufort, N. C., by Dr. H. W. Manter agrees with Price's description on this point. It is believed that the genus *Neoheterobothrium* is justified and the new combination, *Neoheterobothrium reynoldsi* (Frayne, 1943) is proposed.

Family MICROCOTYLIDAE Taschenberg, 1879

*Microcotylodes* n. gen.

*Microcotylodes incisa* (Linton, 1910) n. comb.

(Figs. 9-14)

Synonym: *Microcotyle incisa* Linton, 1910.

**Description:** Body slender, 3.774 to 4.794 mm long (including haptor) by 0.510 to 0.594 mm wide, rounded at anterior end but more pointed at posterior end. Posterior haptor one-third total body length, with 68 to 72 pairs of suckers; suckers 0.076 to 0.086 mm long by 0.044 to 0.053 mm wide. Each haptoral sucker with seven chitinous pieces (Fig. 13): a median U-shaped piece (a) curved dorsoventrally; two pairs of slender curved pieces (b, b' and c, c'); and an anterior pair (d, d'). Mouth subterminal. Oral suckers elliptical, 0.076 to 0.085 mm long by 0.047 to 0.051 mm wide; each provided with a septum; inner edge of oral sucker bordered by an extension of a thin, cuticular membrane the edge of which is armed with numerous minute spines (Fig. 12). Pharynx spheroidal, 0.034 to 0.037 mm in diameter. Testes ovoid, post-ovarian, 20 to 30 in number. Vas deferens a large sinuous tube extending forward to near the atrium where it bends backward to enter a cylindrical, muscular prostatic bulb, 0.076 to 0.085 mm long by 0.025 mm in greatest width (Fig. 10). The male tube leads posteriorly from the bulb a short distance then bends forward dorsally to become continuous with the penis sheath. Two straight, rod-like diagonal tubes, the prostatic reservoirs, converge to enter the base of the penis sheath (Fig. 10). Penis long, slender, containing three rod-like chitinous pieces. Genital pore median, 0.238 to 0.255 mm from anterior end of body. Genital atrium bulb-shaped, non-muscular, without spines. Ovary median, near posterior end of anterior half of body; oötype median, well anterior to ovary. Vitellaria in lateral fields, extending into anterior portion of haptor where they converge medianly. Vagina present. Vaginal pore in right lateral margin of body, approximately 0.36 mm from anterior end. Vagina divided into two regions, the distal portion lined with smooth cuticula; the proximal portion with rough, crinkled lining. Eggs ellipsoidal, 0.212 to 0.221 mm long by 0.113 to 0.169 mm wide with very long, coiled anterior filament (2.0 mm or more, if straightened) and a shorter, uncoiled posterior filament possessing a terminal knob-like enlargement.

This description is based on six specimens.

**Host:** *Lutjanus griseus* (Linnaeus), gray snapper.

**Location:** Gills.

**Localities:** Tortugas, Florida, and Bermuda Islands.

**Slide:** U. S. Nat. Mus. Helm. Coll. No. 36892.

**Discussion.**—Linton's description of *Microcotyle incisa* is very incomplete but it is sufficient to show he was dealing with the above species (from the same host and locality). Linton's figure of the egg is incorrect at least for a typical egg. Eggs become attached to one another by the anterior filament coiling around the short posterior filament of the egg in front (Fig. 14). The knoblike swelling apparently serves to prevent the eggs from separating.

#### Diagnosis of *Microcotylodes*

MICROCOTYLIDAE.—Body flat, elongate; posterior haptor as in *Microcotyle*. Atrial opening median, ventral; genital atrium unspined. Male system with prostatic bulb, two prostatic reservoirs, and slender penis possessing three rod-like chitinous pieces; testes post-ovarian. Vaginal pore in right lateral margin of body. Oötype well anterior to ovary.

**Type species:** *Microcotylodes incisa* (Linton, 1910).

**Comparisons.**—*Microcotylodes* differs from *Microcotyle* chiefly in the lateral position of the vaginal pore, and in the male terminal organs: chitinous penis, prostatic bulb and prostatic reservoirs.



## SUMMARY

Two new species of monogenetic trematodes are described: *Hexostoma macracanthum* and *Cyclocotyla hysteroncha*, from the gills of marine fishes from Tortugas, Florida.

*Choricotyle reynoldsi* is transferred to the genus *Neoheterobothrium*.

A new genus, *Microcotyloides*, is named and described with *M. incisa* (Linton, 1910) as type species.

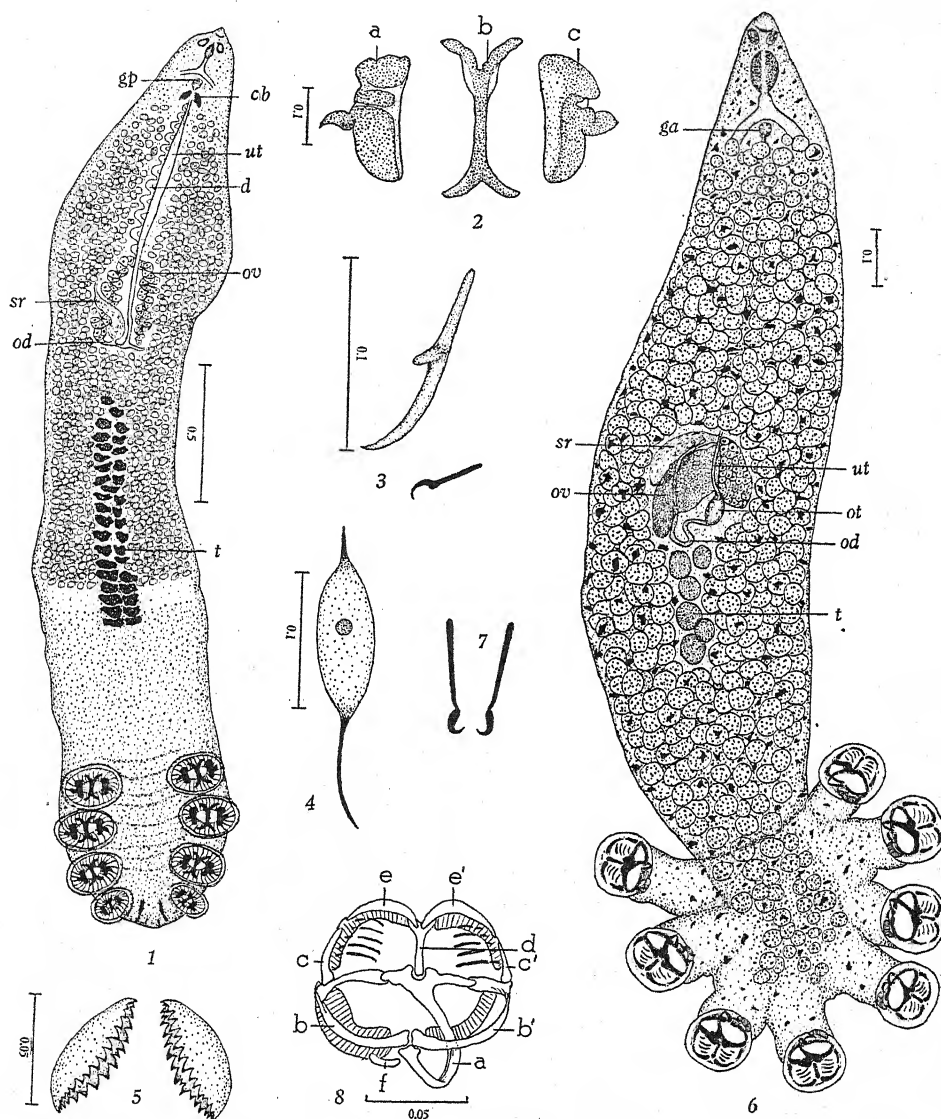
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## EXPLANATION OF PLATES

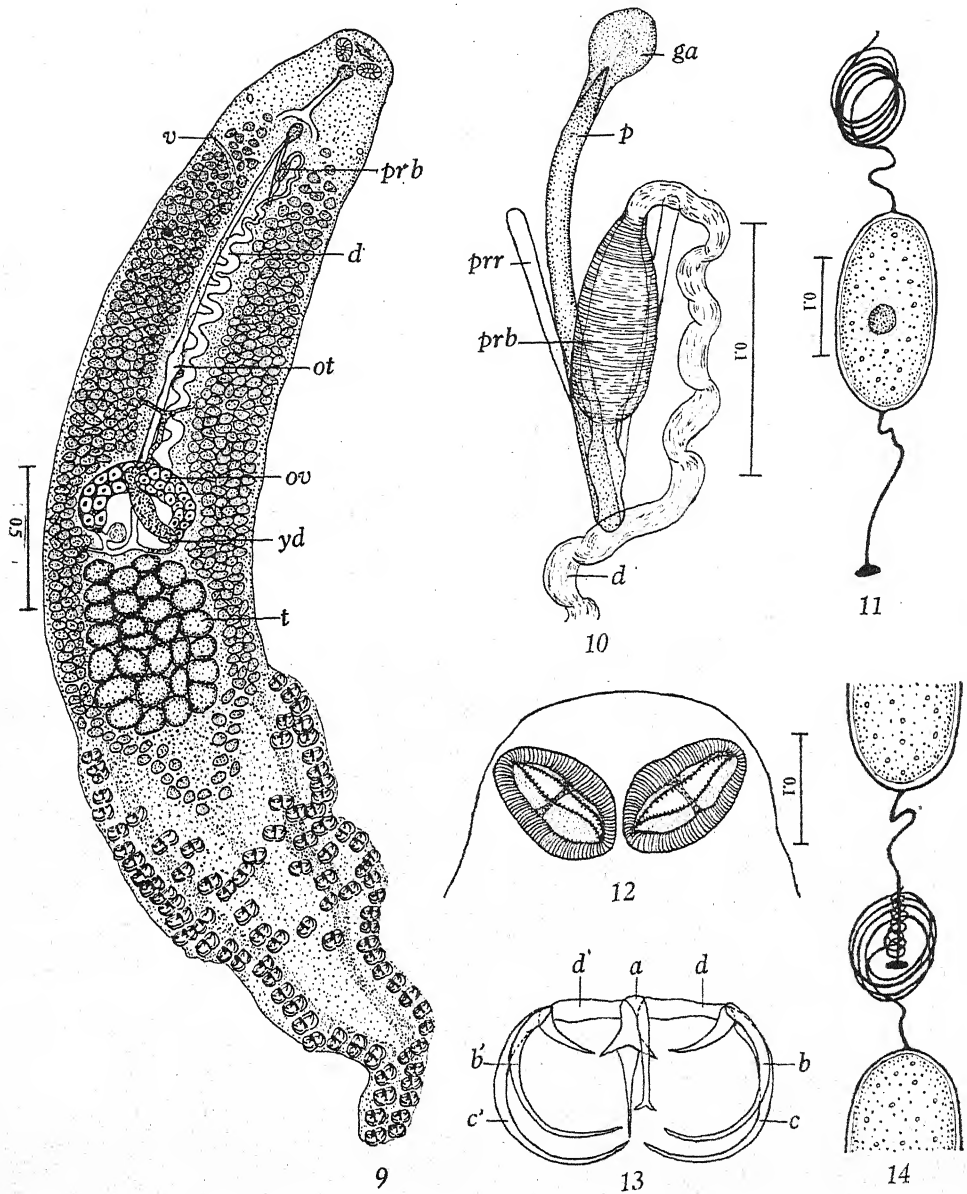
All drawings were made with the aid of a camera lucida. Abbreviations are as follows:

cb	cuticular body	prb	prostatic bulb
d	vas deferens	prr	prostatic reservoir
ga	genital atrium	sr	seminal receptacle
gp	genital pore	t	testis
od	oviduct	ut	uterus
ot	oötype	yd	yolk duct
ov	ovary	v	vagina
p	penis		



## PLATE I

- FIG. 1. *Hexostoma macracanthum*. Ventral view.  
 FIG. 2. Skeletal framework of anteriormost (left) posterior sucker of *H. macracanthum*:  
 (a) inner piece; (b) middle piece; (c) outer piece.  
 FIG. 3. Posterior hooks of *H. macracanthum*, showing left hook of each pair.  
 FIG. 4. Egg of *H. macracanthum*.  
 FIG. 5. Cuticular, denticulated bodies of *H. macracanthum*.  
 FIG. 6. *Cyclocotyla hysteroncha*. Ventral view.  
 FIG. 7. Posterior hooks of *C. hysteroncha*.  
 FIG. 8. Skeletal framework of anteriormost (right) posterior sucker of *C. hysteroncha*.  
 Pieces are labeled as described in text.



## PLATE II

- FIG. 9. *Microcotyloides incisa* (Linton, 1910). Ventral view.  
 FIG. 10. Enlarged portion of male reproductive system of *M. incisa*. Ventral view.  
 FIG. 11. Egg of *M. incisa*.  
 FIG. 12. Oral suckers of *M. incisa*. Ventral view.  
 FIG. 13. Skeletal framework of one of the posterior suckers of *M. incisa*. Pieces are labeled as described in text.  
 FIG. 14. Anterior filament of one egg of *M. incisa* coiled about posterior filament of another.

*SPELOTREMA PSEUDOGONOTYLA* N. SP. (TREMATODA:  
MICROPHALLIDAE) FROM HONGKONG

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In the course of a study of heterophyid trematodes from Hongkong, several specimens belonging to the family Microphallidae were encountered. They are members of the genus *Spelotrema* and differ enough from any known species to be described tentatively as new; the name *Spelotrema pseudogonotyla* is proposed for them.

*Spelotrema pseudogonotyla* sp. nov.

(Figs. 1-4: all measurements in mm.)

The material studied consists of 50 specimens mounted *in toto* and 8 specimens cut in cross, frontal, oblique and sagittal sections. Since living specimens are not available, and since the large number of eggs covers up most of the internal structures, much of the present study was done on serial sections.

The trematode is ovate, flattened dorso-ventrally in the anterior half, often somewhat concave on ventral surface; body 0.385-0.472 long by 0.184-0.230 wide, with an average of 0.207 by 0.425, greatest width being often at posterior half; cuticle spiny, spines less conspicuous towards posterior end.

Oral sucker terminal, 0.026-0.062 long and 0.026-0.059 wide, average 0.044-0.047; acetabulum distinctly smaller, 0.033-0.043 in diameter, average 0.037, located at almost posterior third of body; genital opening large, somewhat oval in shape, placed to left of acetabulum. Mouth, surrounded by oral sucker, opens into a narrow prepharynx, 0.023 long; pharynx globular, 0.025 long and 0.0245 wide; esophagus slender, 0.106 long and 0.009 wide; intestinal bifurcation at about middle of body; ceca large and short, 0.1 long by 0.024 wide, ending in front of or at level of acetabulum.

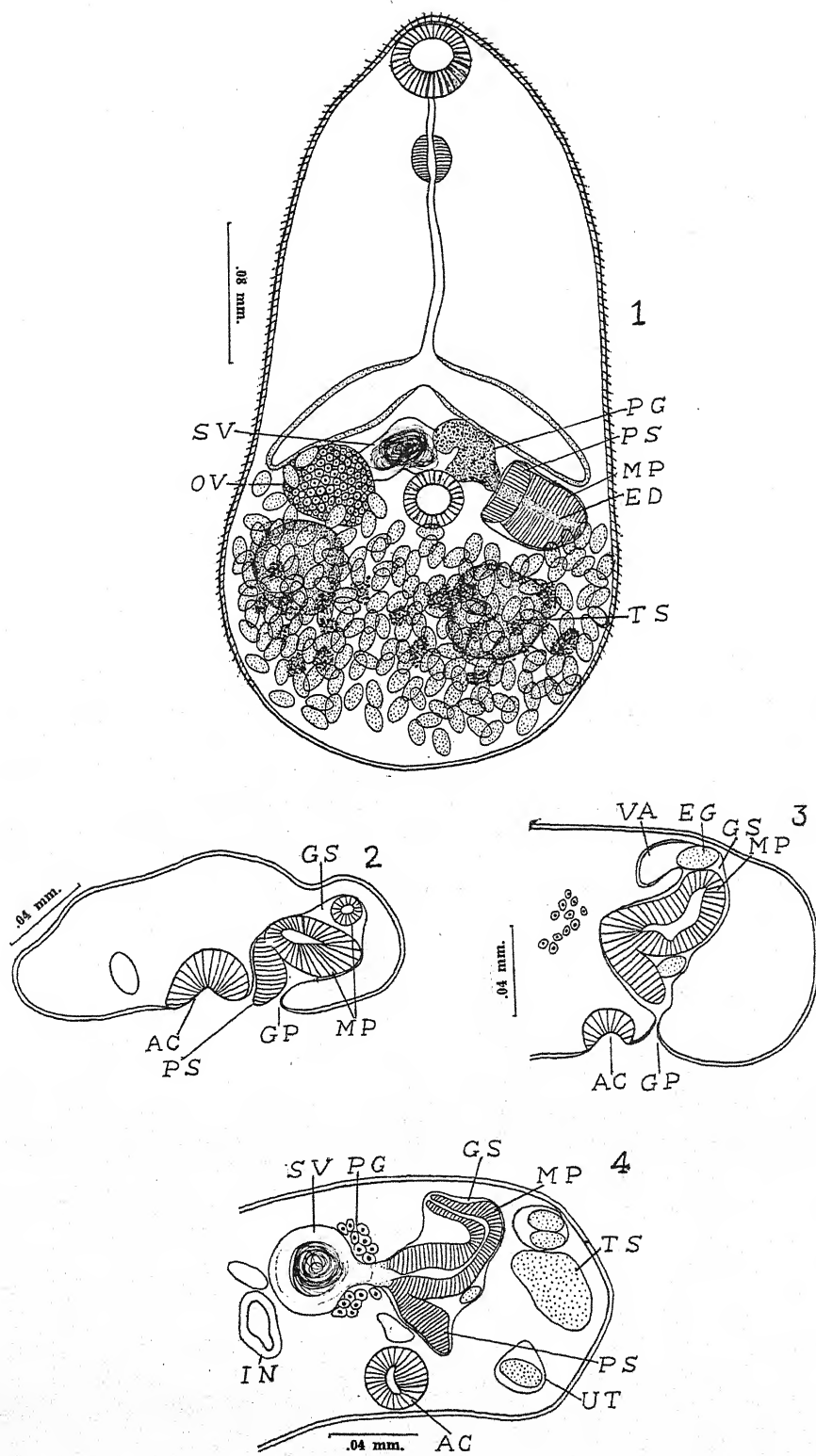
Reproductive organs mostly covered by enormous number of eggs; tests two, about 0.05 in diameter, post-acetabular, lying against dorsal wall of body, somewhat obliquely placed opposite each other. From each arises a vas efferens, left one being longer. A short vas deferens leads to an inverted V-shaped seminal vesicle, 0.056-0.082 by 0.036-0.059, average 0.067 by 0.048, placed conspicuously in front of acetabulum, just behind intestinal bifurcation. Prostate glands well developed, surrounding distal portion of seminal vesicle and the short prostatic section; prostate gland mass measures 0.03-0.049 by 0.014-0.039, average 0.039 by 0.028. The prostatic section continues on to form a small but long ejaculatory duct surrounded by a large muscular structure, the male papilla. The latter is bounded on its ventral side by a large and somewhat curved muscular flap, which guards the entrance of the genital pore and is here termed a pseudogonotyl. The male papilla and pseudogonotyl are fused together at their bases. The male papilla narrows and bends dorsally much like a hook, with the male opening at the end. The genital sac, holding the two muscular structures, is spacious. Viewed either dorsally or ventrally, without taking into consideration the distal curved part, the papilla appears as a broad structure, measuring 0.059-0.076 by 0.04-0.053, average 0.069 by 0.047. However, in sections it is about 0.085 in entire length. The pseudogonotyl from the same views measures 0.04-0.056 by 0.013-0.026, average 0.051 by 0.02.

Ovary oval, 0.049-0.066 by 0.04-0.053, average 0.059 by 0.041, somewhat transversely placed just in front of right testis, lying close against the right cecum, near dorsal body wall at about the same level as acetabulum; seminal receptacle not observed. Oviduct turns downward, and at posterior level of testes it turns upward to become the oötype surrounded by Mehlis' glands. The latter mass measures 0.015 by 0.019, located dorsally somewhat on right side and slightly behind testes. Vitellaria in two groups, ventral to male genital glands, between which are the common vitelline duct and the yolk reservoir. Uterus voluminous, containing numerous eggs, occupying entire posterior region up to the tip of the cecum on the right and the posterior limit of genital sac

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\* The University was re-opened in Kungong, Kwangtung, 1942, after the fall of Honkong to the enemy (originally located in Canton but moved to Hongkong when Canton was invaded in 1938). The manuscript was completed in Hongkong but was only recently smuggled to the Free China. The author is now a guest professor in the Chung Cheng Medical College, Yunghsin, Kiangsi Province, China.





on the left. Proximal end of uterus extends downward from oötype to posterior end of body and then bends upward towards the right, all this portion being filled with spermatozoa. Distal end connected with a short metraterm which opens into the dorsal side of genital sac. Eggs 0.023 by 0.013, oval, embryonated when fully mature, often with a conspicuous rim at operculated end and a minute nodule at opposite end.

Excretory bladder hardly visible in whole mounts. In serial sections it appears V-shaped with a very short stem. Excretory opening terminal.

*Location*.—Intestine.

*Host*.—Domestic ducks (*Anas* sp.)

*Locality*.—Type specimens from Hongkong. The same species was seen by the writer previously in Canton.

*Type and cotype specimens*.—In the Division of Biology, Lingnan University.

The present species can be distinguished from other species of the genus in possessing a special muscular structure, called here pseudogonotyl, which is attached at the base to the male papilla and guards the entrance of the genital pore. If the distinction among the several genera known at the present time in the family Microphallidae is chiefly in the muscular structure of the male system as elucidated by Rankin (1940), then it may become necessary in the future to reconsider the generic standing of this species. This, however, can be avoided at the present time since, as noted by Tubangui and Africa (1938), our knowledge of the group is still incomplete. Another prominent feature of this species is the typical hook-shaped male papilla.

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#### EXPLANATION OF FIGURES

##### Abbreviations

AC—acetabulum	IN—intestine	SV—seminal vesicle
EG—egg	MP—male papilla	TS—testis
ED—ejaculatory duct	OV—ovary	UT—uterus
GP—genital pore	PG—prostate glands	VA—vagina
GS—genital sac	PS—pseudogonotyl	

FIG. 1. Adult worm, drawn from mounted specimen with certain structures added from observations on serial sections.

FIG. 2. Cross section of adult worm, showing the position of the pseudogonotyl in relation to the male papilla and the genital pore.

FIG. 3. Cross section of same worm as Fig. 2, showing connection between the genital sac and the vagina, and relation of the genital pore to the acetabulum.

FIG. 4. Oblique section of adult worm, showing relationships of reproductive structures and the hook-shape of the male papilla. A portion of an egg is seen in the genital sac.

# SEASON, NUTRITION, IMMUNITY, DRUGS AND X-RAY AS FACTORS INFLUENCING THE COURSE OF A COCCIDIAN INFECTION\*

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*Adelina deronis* is a coccidian parasitic in peritoneal cells of the aquatic oligochaete, *Dero limosa*. During the four-year course of a cytological analysis of the life history of this sporozoan parasite (Hauschka, 1943) extensive ecologic and experimental data were accumulated. Some of these results, bearing mainly on host-parasite relationship, are here combined under one title. The ecologic study is confined to statistics on seasonal incidence. The experimental work is concerned with vitamin effects on host-resistance, controlled infection by cyst-feeding, host specificity and immunity and with attempts to check the parasite through anti-malarial drugs and X-ray.

A self-explanatory diagram of the life cycle reprinted from an earlier publication is given in Text-figure 1.

## I. MATERIAL AND METHODS

The seasonal incidence percentages are based on the microscopic examination of 2540 specimens of *Dero limosa* collected over a period of two years from a pond in the University of Pennsylvania Botanical Garden.

Cultures of two species of *Dero* and of five other naid genera (*Nais*, *Aulophorus*, *Pristina*, *Stylaria*, *Chaetogaster*) were grown in a boiled lettuce medium (Hyman, 1941) with or without the addition of measured quantities of eight B-complex vitamins (Hauschka, 1944). Both infected and parasite-free strains of *Dero limosa* were cultured. Each *Dero* clone was derived from a single worm through budding. Genetic identity of all the animals in a given series was thereby assured.

Comparative growth curves, plotted through weekly counts of individuals and somites in the various cultures, established differences in *Dero* fission rate as conditioned by genetic, nutritional and parasitic factors.

Sporulated *Adelina* oöcysts were fed to parasite-free hosts belonging to six different genera of the NAIDIDAE. The small size and great transparency of the hosts as well as their known culture history practically eliminated errors in the subsequent diagnosis of the infection. The time-relations of the various stages in the coccidian life-cycle and several facts concerning host specificity and natural immunity were thus established.

Attempts were made to rid hosts of their infections by checking the parasites with quinine sulphate, plasmochin naphthoate and atabrin. Aqueous solutions of these drugs, corresponding in concentration to human anti-malarial dosages, were employed in addition to more concentrated and weaker solutions.

Twelve infected hosts were irradiated at weekly intervals with unfiltered X-ray (Pecker-Zephyr apparatus, 100 Kv) ranging in dosage from 35 r to 7000 r.

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\* The critical advice of Professor D. H. Wenrich and the expert assistance of Dr. J. T. Farrell, Jr., in the X-ray work is gratefully acknowledged.

Photomicrographs were taken of living parasites through the transparent body walls of chloretonized hosts, without injury to the latter. Exposures ranging from 1/2 to 1/5 sec. were made on 35 mm Kodak Panatomic X film with the aid of a Leitz Micro-Ipso attachment, a Leitz 10× compensating ocular and a Spencer 4 mm achromatic objective. A Sobotka lamp served as light source.

## II. RESULTS

1. *Seasonal incidence.*—Forty-eight collections totalling 2540 specimens of *Dero limosa* were taken from the same location in the above mentioned pond during April, May, October and November, 1942, and April and November, 1943. Every worm was examined individually under the microscope.

A total of 1477 animals collected during the spring yielded only 35 infections or 2.3%. During the fall 340 out of a total of 1063, or 31.9% were infected.

Collections taken in December and January were too small to be statistically valuable. During the warm months of the year the oligochaetes build their mucous tubes mainly on the abundant pond weed *Ceratophyllum*. When this plant dies off during December, it sinks to the lower levels of the pond, and some of the worms probably survive the winter in the bottom debris. The more heavily parasitized hosts presumably perish. Vegetative fission ceases almost entirely at winter temperatures and regenerative capacity is no longer capable of counterbalancing parasitic damage to vital tissues. When warm weather returns the surviving worms are largely unparasitized; but many oöcysts may be available for ingestion.

During the spring and summer months *Dero* reproduces sexually in addition to vegetative budding. Since *Adelina deronis* cannot be transmitted through the minute *Dero* egg, which is considerably smaller than a merozoite, an entire new generation of potential hosts arises, greatly swelling the total of uninfected worms.

In summer the infection is widely disseminated. This is accomplished not merely through ingestion of mature oöcysts. The great rise in incidence from 2% in the spring to 32% in the fall is caused largely by transmission from anterior to posterior zoöid through the fission zone during vegetative budding. Evidence for this is the concentration of parasites in the head region of many of the worms examined. On the other hand, infections resulting from cyst-ingestion are most concentrated in the peritoneum opposite the midgut and anterior hindgut.

The efficiency of parasite-transfer through host budding is best shown in laboratory clones. Forty-two days after isolation of eight infected specimens in eight separate dishes the population had risen to 57 animals. Fifty-one of these were infected.

2. *Nutritional factors.*—The B-complex vitamin requirements of two species of *Dero* are described elsewhere (Hauschka, 1944). Eight infected clones of *Dero limosa*, derived from single individuals through budding, began to die off after two months of culture in a B-complex-deficient lettuce medium. In five similar cultures decline was prevented by the addition of a solution of eight crystalline B-vitamins. In spite of rather heavy infection these clones are still growing after seven months of culture. The vitamins greatly increased the lettuce intake of the hosts and thereby aided in maintaining regenerative potency somewhat above the demands of the parasite, so that the worms could undergo fission besides repairing and replacing damaged cells.



While the vitamin-B effect on host growth was very marked, no qualitative difference in the parasitic life cycle was noticed. Since the initial infectious dosage of oöcysts and migration of merozoites across host-fission zones were not controllable, a quantitative comparison of oöcyst yield in tests and controls could not establish the existence of a vitamin effect. Extremely heavy as well as light infections occurred through six generations of a slow-growing clone fed on a vitamin-deficient diet of scalded lettuce alone. Fast-growing clones receiving the B-complex supplement likewise exhibited a marked range in the severity of their infections. From this apparent absence of correlation it seems that the parasite's growth is not necessarily a function of host growth, although conversely host fission frequency is often a function of the parasite population. (See below.)

The quantitative experiments of Becker and Morehouse (1937) and Becker (1940) conclusively demonstrated vitamin effects on *Eimeria nieschulzi* in the rat intestine. A complex of factors, called "coccidibios" by Becker (1940), enhances *Eimeria* oöcyst production, while vitamin B<sub>1</sub> depresses it. "When B<sub>1</sub> is limited, B<sub>6</sub> has been found definitely to promote oöcyst numbers. It may be that 'coccidibios' is a complex of which vitamin B<sub>6</sub> is one of the factors."

In *Dero* the close balance existing between the destructive parasitic factor and vegetative fission rate is evident from a comparison of two culture series: (a) Eight parasite-free isolation lines, each derived from a single animal, showed the following increase after four weeks: 5, 6, 6, 7, 7, 7, 8, 8 (average 6.75; range 5-8). This served as control for (b) eight infected lines, also derived from single worms, which after four weeks contained 1, 2, 2, 4, 5, 6, 7, 8 individuals (average 4.37; range 1-8). One of the infected worms had failed to divide, two had divided only once in four weeks, while the fission rate of the rest was comparable to the control series. Since the animals with fewest offspring were also the most heavily infected, inverse relation is indicated between severity of infection and fission frequency. On the whole, the growth rate of the parasitized series is only 35% below that of the uninfected control.

Starvation of *Dero* does not detectably affect the life cycle and normal cytology or cyst viability of *Adelina deronis*, but results in a loss of balance between damage and repair and consequent dwarfing and death of the host.

The state of nutrition of a host and, to some extent, the physiological age of a given somite, is often expressed by the condition of the chloragogen tissue, which in *Dero* is homologous to the visceral peritoneum. According to Freudweiler (1905), as quoted by Stephenson (1930), the chloragocytes function in the storage of food reserves which are laid down as greenish-yellow globules. Liebmann (1943) states that chloragogue cells frequently contain guanine and draws attention to the work of Hammett (1938) on the growth-activating properties of this purine base in low concentrations.

In worms heavily infected with *Adelina deronis* and consequently undergoing much internal regeneration, there is an abundance of dark brown chloragogen bodies along the intestine (Figs. 1 and 3). Parasite-free hosts have fewer and less refractile chloragogen globules. The animal photographed in Fig. 2 was uninfected until the day before this picture was taken. Here the chloragogen does not stand out as dark spherules and is considerably less concentrated than in Fig. 3. The recent finding of Liebmann (1943), that regeneration in *Eisenia* is entirely under the control of the chloragogen system, appears substantiated by the regeneration-conditioned variations of this system in *Dero limosa*.

3. *Experimental infection of Dero limosa*.—The average duration of the various stages in the life cycle appears in Text-figure 1. The time-relationship was determined through experimental infection of hosts from negative clones. In about 2/3 of the attempts infection was accomplished by a single feeding with crushed worms containing sporulated *Adelina* oöcysts (Figs. 3 and 7).

Within three to four days after cyst feeding large schizonts are recognizable in the peritoneal cells surrounding the posterior midgut (Fig. 8). Due to their lack of refractile inclusions these trophic forms do not yield satisfactory photomicrographs. The stage marked sch 1, in Fig. 5 is a nearly mature schizont showing over twenty merozoites grouped about a central residual mass. It corresponds to stage 11 in Text-figure 1. Three days after maturing of the first generation schizonts, merozoite bundles of the second generation (sch 2, Fig. 4 and stage 14, Text-figure 1) make their appearance. These smaller schizonts give rise to male and female gametocytes, respectively, mating of which may be observed in vivo. In Figs. 4 and 9 recently associated gametocyte pairs predominate (see also stage 17, Text-figure 1). Several somewhat older pairs and trios (2 ♂ attached to 1 ♀) are pictured in Fig. 10. The female in Fig. 5 distinctly reveals its nucleus and nucleolus against the more refractile background of paraglycogen granules in the cytosome.

Fig. 6 represents the condition immediately succeeding fertilization. The nucleus on the lower left is a synkaryon. The microgametocyte remnant on the upper right has been slightly pushed away from the zygote by the recently formed fertilization membrane which in due course gives rise to the oöcyst wall (Fig. 3). Mature oöcysts are usually present 16–18 days after ingestion of the original infectious dose. Oöcysts can hatch in situ and thus the life cycle is reopened without transfer to a new host. For a discussion of the self-limited nature of this cycle the reader is referred to an earlier paper (Hauschka, 1943).

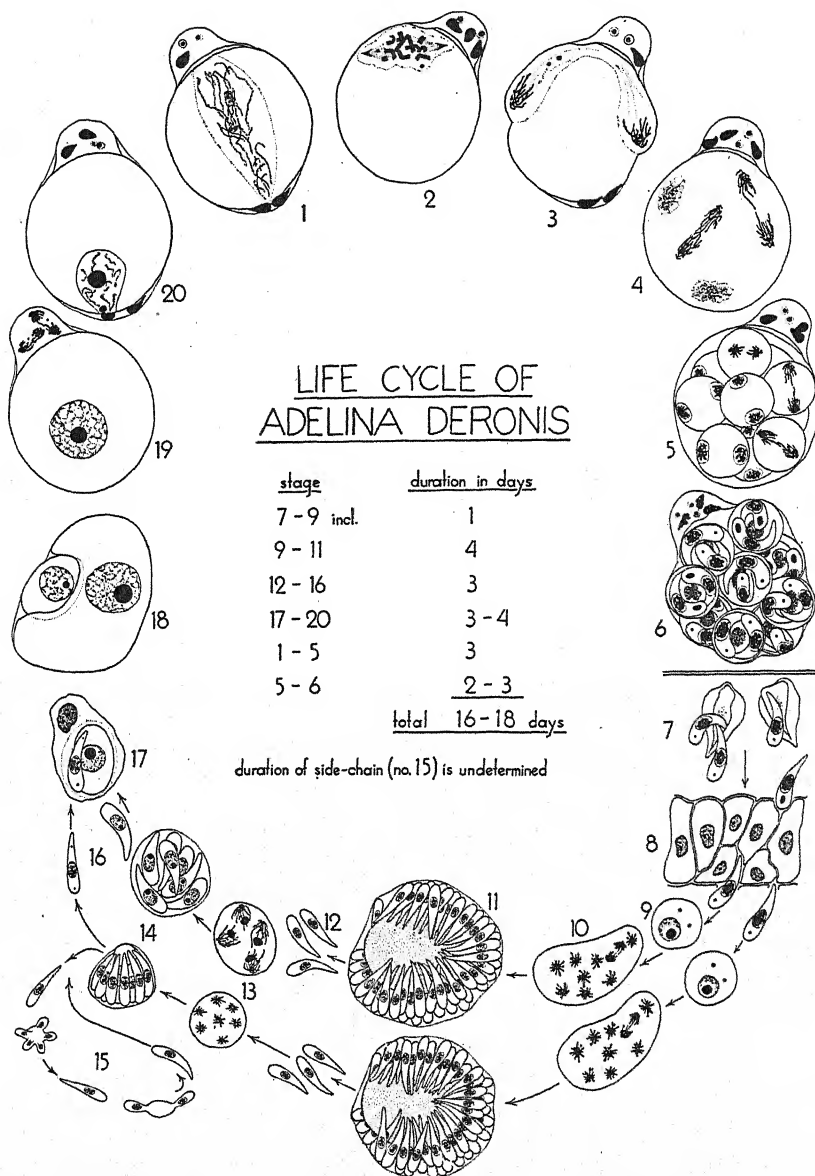
4. *Host specificity and immunity*.—In addition to negative clones of *Dero limosa* several entire cultures and thirty-seven isolated individuals of a larger, unidentified *Dero* species were repeatedly exposed to the infection. The latter species was clearly distinct from *Dero limosa* in size (being about 2.5 times longer and thicker), in length of setae, average number of somites, growth rate and sexual cycle.

This *Dero* must be considered naturally immune to *Adelina deronis* since continued experimental cyst feedings failed to produce positive results and since none of the many freshly collected specimens were infected.

Likewise unsuccessful were attempts to infect five individuals of *Nais* sp., four of *Aulophorus* sp., twenty-two of *Chaetogaster* sp., sixteen of *Pristina longiseta*, a large culture of *Stylaria lacustris* and a culture of *Nais paraguayensis* (available through the kindness of Dr. L. H. Hyman). The oöcysts were readily ingested by many of these oligochaetes, except *Chaetogaster*.

Even within the species *Dero limosa* experimental infection does not always succeed. In spite of heavy dosages of sporulated oöcysts, six out of sixteen infection attempts gave negative results.

To determine whether this relatively high percentage of failure was caused by inadequate procedure, an apparently uninfected specimen was isolated and the culture was quarantined for two weeks when it contained four genetically identical worms produced by budding. These animals were parasite-free and they and their offspring remained negative for seventy days during which period large numbers of oöcysts were fed to them on five successive occasions.



*Explanation of Text-figure 1*

1. Zygote, showing homologous chromosomes in process of synapsis.
2. Metaphase of meiotic division of synkaryon.
3. Zygotic meiosis, telophase; this division is immediately followed by a second meiotic division.
4. Early sporogony; karyokinesis not synchronous; this is possibly indicative of the primary sex difference.
5. Late sporogony.
6. Mature oöcyst (double line between 6 and 7 indicates change of host).
7. Spores hatching in host gut; spores are thought to produce male and female sporozoites respectively.
8. Sporozoites penetrating midgut epithelium (stages 7 and 8 may be by-passed in auto-infection).
- 9, 10, 11. First schizogonic generation, during which no sex difference is apparent.

The actual ingestion of cysts and the hatchings of spores in the intestine were observed microscopically. A photomicrograph (Fig. 2) obtained on the twenty-ninth day of this experiment, shows a sporulated oöcyst (oö) and a recently hatched sporozoite (sp) within the host-gut. The exposure time of 1/5 sec. was not rapid enough to counteract slight blurring due to the twisting movements of the parasite, which may be seen rather faintly near the end of an ingested bristle.

Motile sporozoites were repeatedly observed also in the coelomic fluid of most of the worms in this series, but the half-grown trophic stages of the parasite corresponding to stages 9 and 10 in Text-figure 1 appeared to degenerate within the host cells. None of them ever developed into bundles of merozoites. Instead, they became filled with brownish green globules resembling chloragogen and after a few days were completely replaced by this material.

These happenings in immune host cells, as observed in vivo, correspond with the findings of Tyzzer, Theiler and Jones (1932) in chickens immunized through successive feedings with *Eimeria necatrix* oöcysts. A cytological study of the intestinal mucosa of these birds revealed that "most of the sporozoites are destroyed after invading the gland cells by the failure of the latter to respond in such a way as to favor the growth of the parasite. No evidence has been obtained of any protective mechanism attributable to humoral response."

The observations recorded below in a day by day account were made on lightly chloretonized hosts at a magnification of 400 diameters. After each examination the worms were allowed to recover in spring water and were returned to the culture dish. No animal in this series was lost through injury:

- Day 1. A single negative *Dero limosa* isolated in lettuce medium in Syracuse watch glass.
14. Four worms in culture; all negative.
- 14-16. *First exposure* to cysts from three heavily infected hosts; empty sporocysts observed in gut.
17. *Second exposure* to cysts from seven heavily infected hosts.
18. Oöcysts and sporocysts in gut.
24. All worms negative. One degenerating trophozoite in coelom of one of the animals.
28. All worms negative.
28. *Third exposure* to cysts from ten positive hosts.
29. Oöcysts, sporocysts and free motile sporozoites in host-gut seventeen hours after feeding.
29. *Fourth exposure* to cysts from ten positive hosts.
32. Motile sporozoites in coelom of three hosts; several degenerating trophozoites.
35. Only one host with two motile sporozoites and degenerating trophozoites in coelom. Others negative.
53. Six worms in culture, all negative.

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12, 13, 14. Second schizogonic generation, during which sex is differentiated.

15. Waning of microgametocyte-producing schizogony.

16. Young gametocytes; the smaller male shows terminal granules and lacks endosome.

17. Young gametocyte pair in host cell.

18, 19. Growth of gametocyte pairs, and production of microgamete nuclei.

20. Fertilization; one of the four microgametes entering maternal nucleus at fertilization pole.

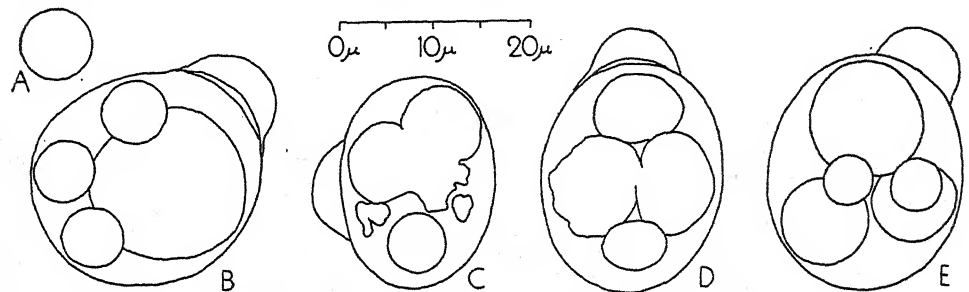


- 54-56. *Fifth exposure* to cysts from two heavily infected hosts.  
 57. One worm with degenerating trophozoites. Five worms with altogether eighteen motile sporozoites in coelom.  
 70. Nine worms in culture, all negative. No traces even of degenerating parasites. Culture discontinued.

Failure to infect this clone is probably not a matter of dietary deficiency. The culture increased by 900% during the seventy days of observation and was resistant to the infection from the outset. Deliberately starved, parasite-free worms from another strain became infected after only one cyst feeding.

The above clone of *Dero limosa* may therefore be considered immune to *Adelina deronis*. The immunity seems to be natural rather than acquired. In susceptible clones transfer of the parasite from host-generation to host-generation continues unchecked over long periods. The repeated inheritance of the infection does not appear to be accompanied by a gradual decline of the parasitic population.

*Adelina deronis* is, thus, not merely limited to one host species, but exhibits strain-specificity within this species, as well as marked tissue specificity, since it invades mainly cells of the visceral and parietal peritoneum.



Explanation of Text-figure 2

Oöcyst aberrations induced by X-ray. A. Normal spore. B, C, D, E. Aberrant spores within oöcysts of normal size.

5. *Effects of three anti-malarial drugs.*—Attempts to check *Adelina deronis* through keeping the hosts in solutions of quinine sulphate, atabrin and plasmochin naphthoate were unsuccessful. A large range of concentrations was tried using human anti-malarial dosages as a basis. In most of these solutions the differentially susceptible heads and tails of the oligochaetes degenerated, leaving only the mid-body region with its contained parasites intact. Host fission was greatly reduced or ceased altogether, while the coccidian schizogony and the development of subsequent parasitic stages continued unchecked in twenty-four experiments. Oöcysts were still viable and caused experimental infection after a sixty-hour soaking in a saturated aqueous bath of plasmochin naphthoate which had killed ten infected hosts in from one to three hours. Of the spores, formed while the hosts were immersed in a non-lethal plasmochin medium, 19.1% were of abnormally large size. In controls such aberrations in sporogony amount to less than 10% of total spores formed.

6. *Effects of X-ray.*—Irradiation of four infected worms with 35 r had no apparent effect on sporogony. All the oöcysts in these animals were normal four days after treatment. In three worms rayed with 1000 r, 35.2% of the oöcysts were aberrant one week after exposure. In four hosts rayed with 5000 r the following

incidence of abnormal spore-formation was observed one week later: 67.3%, 77.0%, 89.2%, 100%. The degree and type of aberration is evident from a comparison between Text-figure 1, stage 5 (normal oöcyst) and Text-figure 2.

Seven non-irradiated control-hosts from the same clones, examined at varying intervals after isolation, contained only 3.4% aberrant oöcysts. The above percentages are based on an examination (in vivo) of 474 irradiated and 619 control oöcysts.

Since schizonts continue to appear quite normal and since spore aberrations are first noticed 3–4 days after irradiation, the most susceptible stage is probably the zygote. During early zygotic synapsis the chromosomes are very long drawn out (stage 1, Text-figure 1) and the chances for effective X-ray hits are thereby greatly increased.

A cytological study of this material, now in progress, should reveal whether the unusually large spores are multi-nucleate, polyploid or aneuploid, and whether the few naturally occurring aberrations involve mitotic pictures similar to the experimentally produced ones.

Abnormal oöcysts from irradiated worms may not be viable. Feeding of a large quantity of them to three negative hosts from a susceptible clone did not produce an infection.

Irradiation between 1000 and 5000 r completely inhibits host fission, while control-worms continue to divide. Five of seven hosts irradiated with 7000 r died within two weeks after treatment and probably as a result of it. Preceding death these animals appeared dwarfed.

### III. SUMMARY

1. In collections of *Dero limosa* gathered during spring only 2.3% of the hosts were infected with *Adelina deronis*. The incidence rose to 31.9% in the fall.
2. Fission frequency of parasitized clones is only 35% below that of controls.
3. A diet of scalded lettuce does not supply the B-complex vitamins necessary to maintain infected cultures over indefinite periods. The parasites in starved hosts appear normal.
4. A correlation between amount of chloragogen, degree of parasitism and tissue repair is indicated.
5. Experimental feeding of sporulated oöcysts to negative host-clones produced infections in 2/3 of the cases. The time relationship and average duration of the various phases of the coccidian life cycle were established.
6. Host specificity is marked as shown by cross-infection experiments involving oligochaetes of the same genus and five other naid genera.
7. Resistance of one clone of *Dero limosa* to heavy infectious dosages and degeneration of trophozoites in the peritoneal cells of this clone is interpreted as due to natural immunity.
8. Quinine sulphate, plasmochin naphthoate and atabrin do not detectably affect schizonts or gamonts, even at concentrations damaging to host tissues. Plasmochin doubles the frequency of abnormal sporogony.
9. Unfiltered X-ray of 1000–7000 r interferes with host fission and produces aberrant sporogony in from 30–100% of the total zygotes. No effect of X-ray on schizogony or gametogony can be observed in vivo.

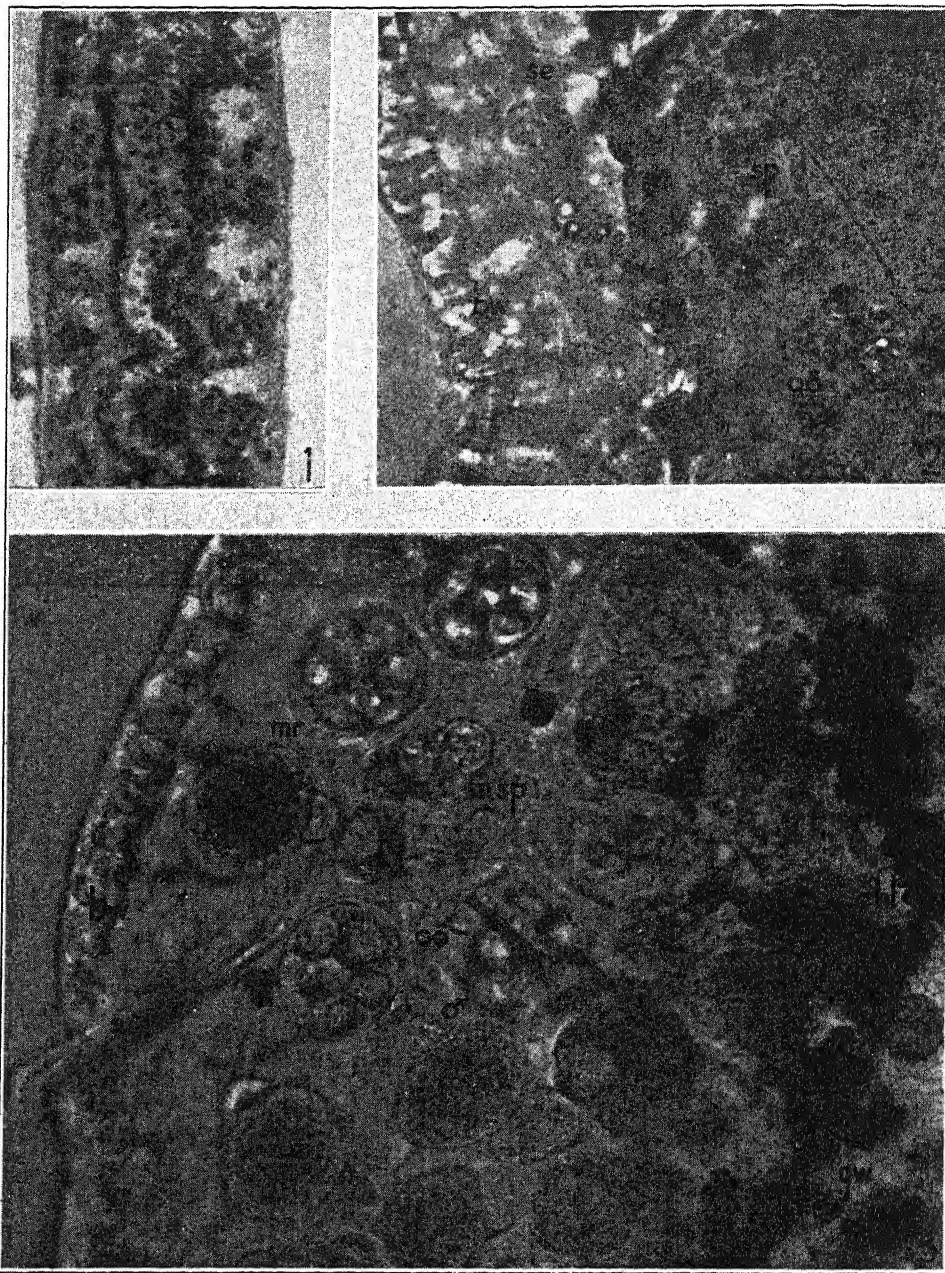


PLATE I

Photomicrographs of the coccidian *Adelina deronis* parasitic in the body cavity of the naid *Dero limosa*. All photographs are of living material, anaesthetized with 0.3% chloretone. Fig. 1 is magnified approximately 130 diameters. In all other figures the magnification is 900 diameters.



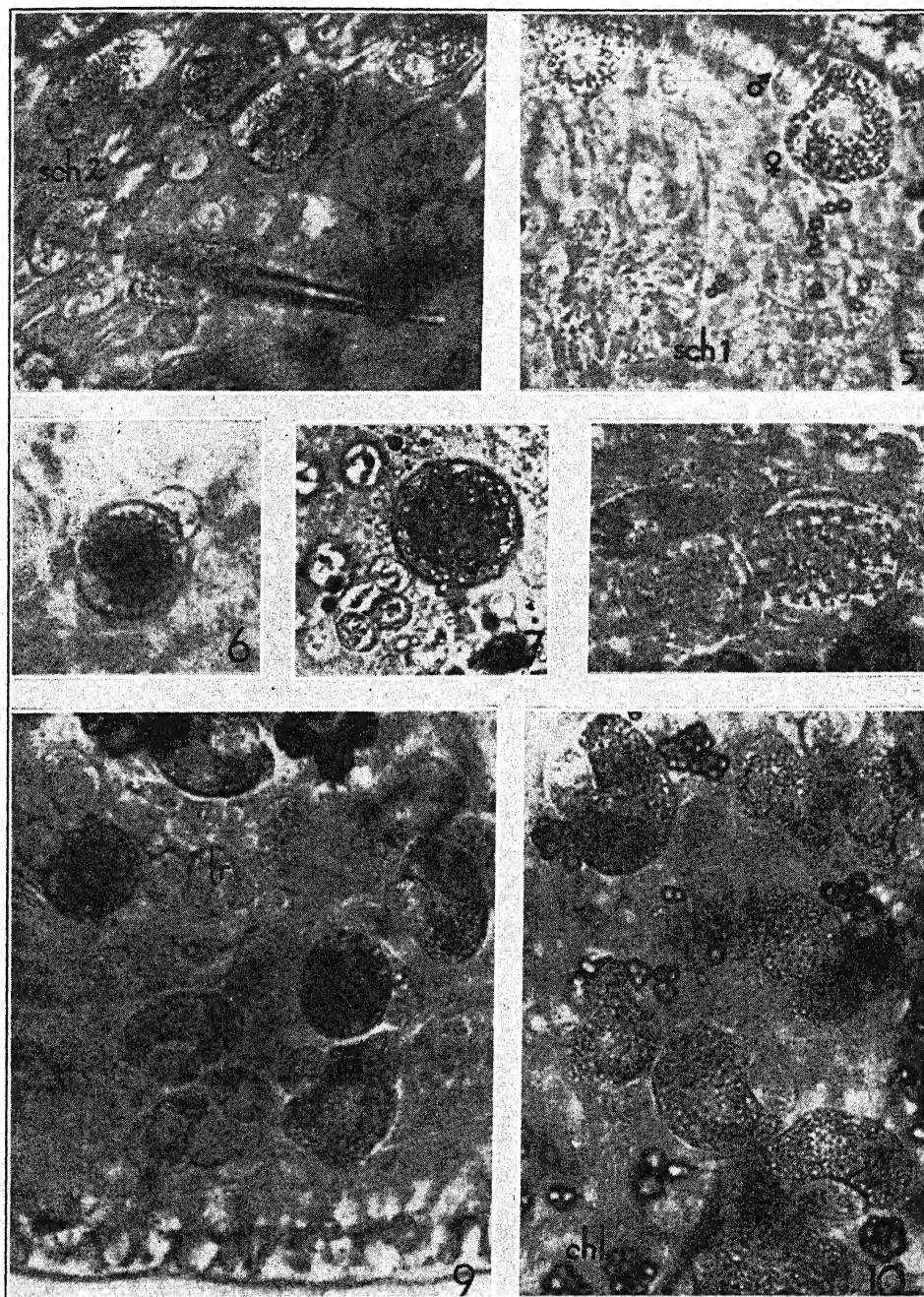


PLATE II

Photomicrographs of the coccidian *Adelina deronis* parasitic in the body cavity of the naid *Dero limosa*. All photographs are of living material, anaesthetized with 0.3% chlorethane. The magnification is 900 diameters.



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*Explanation of figures, Plate I*

FIG. 1. Portion of anterior third of a heavily parasitized worm; dark margin of oesophagus clearly distinguishable due to dense chloragogen material.

FIG. 2. Experimental infection; a newly hatched sporozoite (sp) may be seen free in the intestine; an ingested bristle is pointing to this sporozoite; oö = a recently ingested mature oöcyst, gw = the gut wall, se = a septum, bw = the body wall of the host.

FIG. 3. Appearance of coelom in advanced heavy infection; bw = the body wall, oö = a mature oöcyst, mr = a microgametocyte remnant still attached to mature cyst, msp = mature sporocysts, chl = dark chloragogen granules, gw = gut wall of host.

*Explanation of figures, Plate II*

FIG. 4. Gametogony and pairing; many young stages in peritoneal tissue; sch 2 = a second generation schizont.

FIG. 5. Half-grown gametocyte pair; sch 1 = a mature first generation schizont.

FIG. 6. Zygote shortly after fertilization.

FIG. 7. Mature sporocysts, each containing two sporozoites and a residual body.

FIG. 8. Half-grown schizonts of the first generation; merozoites are beginning to bulge out.

FIG. 9. Young gametocyte pairs; tr = a trophic pair shortly after association.

FIG. 10. Nearly mature gametocyte pairs and trios; chl = chloragogen granules.

A NEW SPECIES OF *RETORTAMONAS* (PROTOZOA) FROM  
THE COMMON MOLE CRICKET, *GRYLLOTALPA*  
*HEXADACTYLA*

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In 1879 Grassi described a species of *Retortamonas*, *R. gryllotalpae*, from the European mole cricket, *Gryllotalpa gryllotalpa*. The taxonomy of these flagellates was reviewed and summarized by Wenrich in 1932. No new species has been recorded since then.

The following observations concern a new species which is named *Retortamonas wenrichi*, in honor of Dr. D. H. Wenrich, who has contributed so much to our knowledge of these small protozoa.

MATERIAL AND METHODS

The host from which this species came was an adult male common mole cricket, *Gryllotalpa hexadactyla*. He was caught on August 29, 1942, as he sang lustily in a chamber of his burrow some eighteen inches from the waters of the Patuxent River, at a spot known as Brown's Bridge, Ednor, Maryland. He was transported alive to the University of Pennsylvania, where these observations were made.

At the time, it was not possible to study the living flagellates extensively, so the present data are all recorded from the permanent slides. The material from the rectum was fixed in Schaudinn's fluid, to which was added 5 per cent of glacial acetic acid. The staining was done in Heidenhain's hematoxylin.

OBSERVATIONS

*The trophic stages.*—The organisms were typical retortamonads. Perhaps the most striking feature of this new form was its extreme length. The longest species described thus far have an over-all range up to 18  $\mu$ . The present species has a relatively large body, and a remarkably long, tenuous tail. The body proper ranges from 12.2  $\mu$  to 19.0  $\mu$  in length, averaging 16.0  $\mu$ . Its width range is 3.8  $\mu$  to 8.4  $\mu$ , averaging 5.9  $\mu$ . The tail ranges from a short spike of 3.1  $\mu$  to a long filament of 58.5  $\mu$ , and averages 23.2  $\mu$ . The over-all length, not including the flagella, ranges from 16.7  $\mu$  to 76.0  $\mu$ , with an average length of 39.2  $\mu$ . That this caudal extension was not in the nature of a stiff spike was demonstrated by the fact that it was frequently looped and twisted (Figs. 3, 5).

The anterior end was often seen to terminate in a short, conical projection (Figs. 2, 5). Tailless, precystic forms were occasionally noted (Fig. 6). Many specimens showed a clear area on one side near the anterior end (Figs. 1-5), which was associated with the oral pouch. Some individuals showed the spirally twisted body (Fig. 4), so common in some species of *Chilomastix*.

The mouth area was bordered on one side by the so-called parabasal fiber, and on the other side by the parastyle. These fibers frequently appeared somewhat thicker near their points of origin (Figs. 2, 4). The parabasal fiber, frequently following a

wavy course (Figs. 3, 4, 5), extended to about the middle of the body proper, then swung across, and often continued some distance back toward the anterior end. This fiber was sometimes accompanied by another stainable thread (Figs. 2, 3, 5), as shown by Geiman (1935) for *Chilomastix* from man, a monkey, and the guinea pig.

The two approximately equal flagella were generally longer than the body, often one and one-half to two times the length of the body proper. Neither showed a marked association with the oral pouch. Arising from separate basal granules, the more anterior one was generally directed forward, while the other frequently took a more backward course, sometimes extending for a short distance along the oral pouch before curving out (Figs. 2, 4, 5). The basal granule giving rise to the second flagellum was also the point of origin for the oral fibers (Figs. 1, 2, 4) but these associations were not always easy to discern.

The nucleus, located very close to the anterior end, was quite like that described by Geiman (1932) for *R. caudacus*, from a gyrenid larva. There was frequently considerable stainable material on the nuclear membrane, and a cloud of stainable material occupied most of the center (Figs. 1-6).

*The cysts.*—These bodies ranged from 6.1  $\mu$  to 8.4  $\mu$  in length, averaging 7.3  $\mu$ . The width was from 4.6  $\mu$  to 6.1  $\mu$ , with an average of 5.1  $\mu$ . Roughly oval and tapering toward the anterior end, they showed a definite cyst wall. As noted by Hegner and Schumaker (1928) in *R. bradypi*, and by Hogue (1933, 1936) in several races of *R. intestinalis*, the cysts of *R. wenrichi* showed a thickened, deeply staining cap (Figs. 7, 8, 9, 10, 11). Even when not especially darkly stained, this greater thickness was in evidence. Unlike the situation as described by Hogue, in which the presence of acetic acid in the fixative prevents the appearance of the cap, those on *R. wenrichi* were quite prominent in the presence of this reagent.

The anteriorly located nucleus was darkly stained, and a tangle of fibers and threads extended posteriorly in the cysts. Anterior lighter areas, similar to those in the trophic stages, were common (Figs. 7, 8, 9, 10, 11).

A unique phenomenon apparently associated with the formation of the cyst wall was observed. In the most recently commenced encystment, the cap appears already established, but without much of the cyst wall (Fig. 7). Soon after this, a faintly staining layer appears about the whole animal (Fig. 8). The cyst wall now seems to be laid down in a progressive anterior-to-posterior fashion, on the innermost aspect of this layer (Fig. 8, wall about half way down; Fig. 9, wall complete except for the posterior region). This peculiar layer persists for a time after the cyst wall is complete (Fig. 10), but eventually disappears (Fig. 11).

FIG. 1. Very long specimen. Viewed from aboral surface.

FIG. 2. Typical retortamonad. Note apical peak, two basal granules, additional oral fiber accompanying the parabasal fiber, and the short course of the more posterior flagellum in the oral pouch.

FIG. 3. Note the thread accompanying the parabasal fiber, and the bent tail.

FIG. 4. Note spiral twist in body.

FIG. 5. Note apical peak, curved tail.

FIG. 6. Precystic form.

FIG. 7. Early cyst, with cap and no enveloping layer.

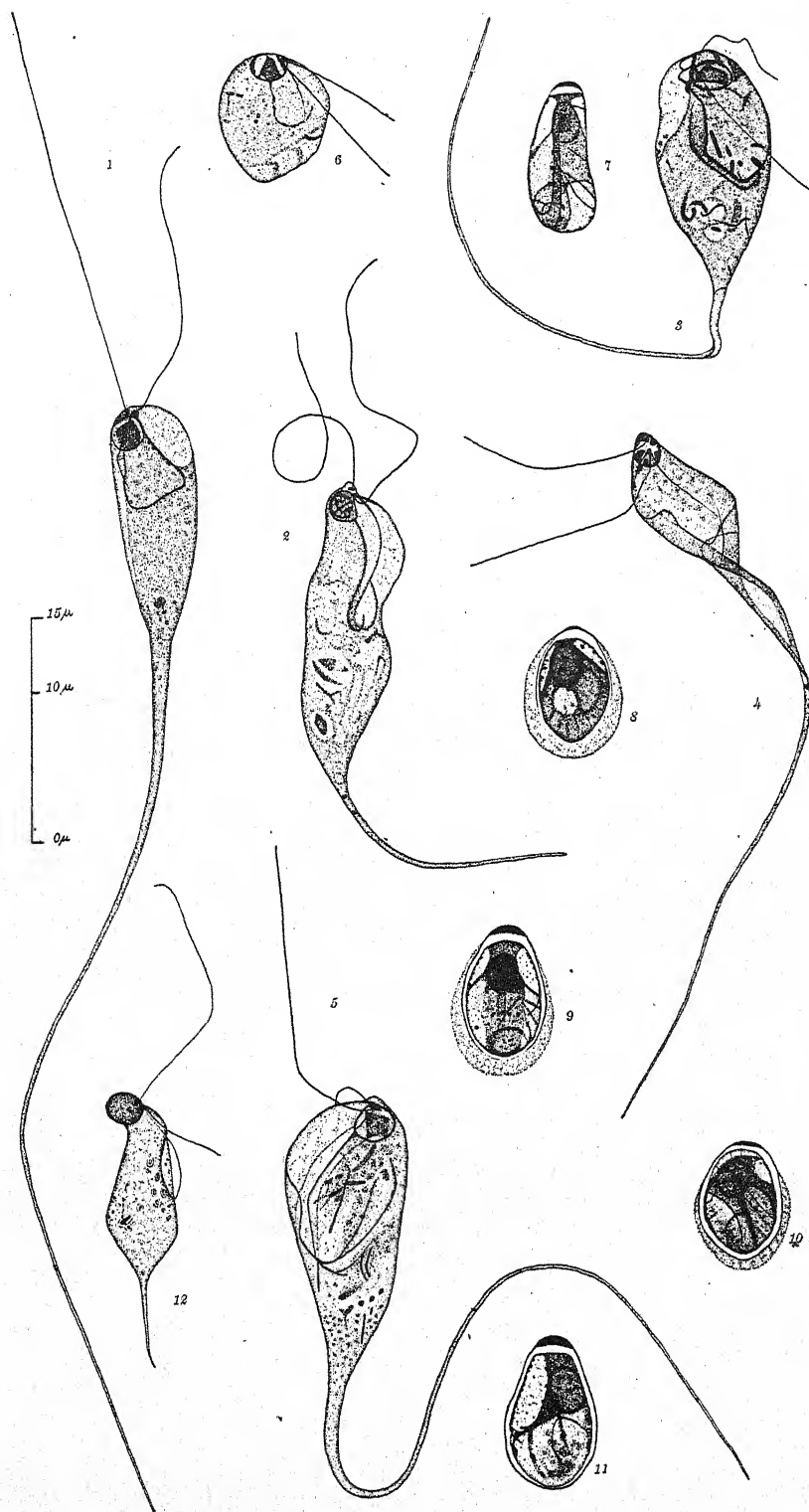
FIG. 8. Layer present, and cyst wall laid down in anterior region.

FIG. 9. Cyst wall nearly complete.

FIG. 10. Wall complete, with layer persisting.

FIG. 11. Wall complete, with layer gone.

FIG. 12. *R. caudacus*, redrawn from Geiman, 1932.



All figures are of *Retortamonas wenrichi*, n. sp., except number 12, which is *R. caudacus* redrawn from Geiman, 1932.



## DISCUSSION

In general body form *R. wenrichi* more closely resembles Grassi's *R. gryllotalpae*, likewise from a mole cricket, and Geiman's *R. caudacus*, from a gyrid larva, than any other species. The extreme length and the nature of the cysts, however, would be ample evidence for the creation of the new species. The formation of the peculiar layer about the encysting forms is a feature not heretofore described for these flagellates.

## SUMMARY

1. A new species of *Retortamonas*, *R. wenrichi*, is described from an adult male common mole cricket, *Gryllotalpa hexadactyla*, collected in Ednor, Maryland.

2. Its most distinctive features are: (a) its extreme length (up to  $76.0\mu$ ), (b) the consistent presence of a thickened, more deeply staining cap on the cyst wall, and (c) the presence of a thin, lightly staining layer about the cystic animal, apparently associated with the formation of the cyst wall.

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## EXOERYTHROCYTIC STAGES OF *PLASMODIUM DURAE*<sup>1</sup>

MYRON L. SIMPSON

In July, 1941, Herman reported the discovery of a new species of avian malaria, pathogenic to turkeys, and named it *Plasmodium durae*. In November, 1941, a duck infected with this species was secured and biological studies were started.

It proved impossible to secure turkey poults at the time the infected duck was obtained, therefore two 13-week-old turkeys were bought from a local breeder and were inoculated intravenously with 3 cc and 5cc, respectively, of blood, obtained by heart puncture, from the infected duck. It was felt that the rather large doses were necessary because of the age of the recipient animals. Nothing is known about the previous parasite history of these two turkeys, but blood examination at the time of their inoculation with *P. durae* was negative for parasites. Both turkeys were inoculated on November 14, 1941, and, in each case, parasites were first seen in blood samples eleven days later. Both birds continued to show parasites until their deaths.

Turkey No. 2 (Table 1) died after 26 days. This animal seemed to be partially paralyzed for several hours before its death, and although it was able to flap its wings, it could not raise its head or stand on its legs. An autopsy was performed and tissue smears made from the spleen, liver, lungs, and brain. These were stained with Giemsa. In smears from all of these organs, numerous bodies were found which were thought to be exoerythrocytic stages of *Plasmodium durae*. They were especially numerous in the lungs and brain, being present in the latter organ in such numbers as to occlude completely many of the capillaries. Some of these bodies were in a comparatively early stage of development, with only one or two nuclei, while many others were in later developmental stages, some apparently approaching segmentation. Other investigators in this laboratory who had had experience with exoerythrocytic stages, including Dr. Robert W. Hegner, Dr. Fruma Wolfson, and Miss Evaline West, were unanimous in the opinion that these bodies were unquestionably the exoerythrocytic stages of *Plasmodium durae*.

Turkey No. 1 (Table 1) was killed 78 days after inoculation; tissue smears of spleen, liver, lungs and brain were made and carefully examined, but no typical exoerythrocytic stages could be found in any of these organs.

Subsequently, as shown in the table, out of a total of 8 young (5-10 days old) turkeys which were inoculated intravenously with *P. durae*, two have been found to have exoerythrocytic stages in the spleen, liver, lungs and brain. Bird No. 4, which was 8 days old, was injected with 0.3 cc of inoculum from bird No. 1. The infected poult died 15 days later and tissue smears demonstrated the presence of exoerythrocytic stages in the usual sites. Bird No. 6, which was 10 days old, was injected with 0.8 cc of inoculum from a duck previously infected. This poult first showed parasites the next day, was patent for 10 days, remained latent for 5 days, then had a relapse and died 23 days after the initial infection. Tissue smears showed the presence of exoerythrocytic stages in the spleen, liver, lungs and brain.

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<sup>1</sup>From the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. The author is grateful to the late Dr. Robert W. Hegner for help in the early stages of the investigation, and to Dr. Fruma Wolfson for assistance in the preparation of this paper.

TABLE 1.—*Exoerythrocytic stages of Plasmodium durae in turkeys*

Bird No.	Age of bird	Size of inoculum, cc	Sub-patent period, days	Tissues examined					Survival after inoculation, days	Fate of bird
				Spleen	Liver	Lungs	Brain	Bone marrow		
1	13 wks.	3.0	11	-	-	-	-	*	78	Killed
2	13 wks.	5.0	11	+	+	+	+	*	26	Died
3	5-10 days	0.3	7	-	-	-	-	*	9	Died
4	5-10 days	0.3	5	+	+	+	+	*	15	Died
5	5-10 days	0.3	4	-	-	-	-	*	13	Died
6	5-10 days	0.8	1	+	+	+	+	*	23	Died
7	5-10 days	0.8	1	-	-	-	-	*	61	Killed
8	5-10 days	1.0	7	-	-	-	-	*	38	Died
9	5-10 days	0.7	7	-	-	-	-	-	50	Killed
10	5-10 days	0.6	7	-	-	-	-	-	50	Killed

\* = smear of bone marrow not made.

+ = exoerythrocytic stages found.

- = exoerythrocytic stages not found.

Of the ten animals reported on, six died as a result of the experimental infections and four were killed at various intervals after the original infection. Exoerythrocytic stages were found in three of the six birds which died of the infection, but not in any of those which were killed. Smears of bone marrow were made in only three of the birds autopsied, but in none of them were the exoerythrocytic stages discovered.

In November, 1942, Purchase reported on a number of natural and experimental infections of *P. durae* in turkeys and other fowl. He stated that schizonts were frequently found in the endothelial cells lining the small blood vessels of the livers, spleens and brains of the experimentally infected birds. They were rarely seen in the lungs. It is of interest to note that in our birds we found the exoerythrocytic stages to be especially numerous in the lungs and brain, although it was not difficult to find them in considerable numbers also in the liver and spleen. It is quite possible that Purchase's "schizonts" and our "exoerythrocytic stages" are identical.

It should also be reported that at least 15 ducks, of various ages, infected intravenously with *P. durae*, were routinely studied in this laboratory. Since, as a general rule, the infection was not fatal to the ducks, they were killed at various intervals after infection, autopsied and tissue smears made. These also were stained with Giemsa and were carefully examined. However, in no instance have exoerythrocytic stages been demonstrable.

Of two old and eight young turkeys infected with *Plasmodium durae*, three were shown to have exoerythrocytic stages in the spleen, liver, lungs and brain. This evidence, while not exhaustive or complete, is believed to be sufficient to demonstrate that exoerythrocytic stages of this newly-discovered avian malaria parasite can and do occur.

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## TRANSVERSOTREMA HAASI, A NEW FISH TREMATODE

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In a basin containing fish brought from the Red Sea about a dozen trematodes, which doubtlessly were parasites of one of these fishes, were found. As there were about twenty species of fish, it was not possible to determine the host. On investigation the specimens proved to belong to a new species of quite unusual structure, not fitting into any known genus or family. It is proposed to name it *Transversotrema haasi*.<sup>1</sup>

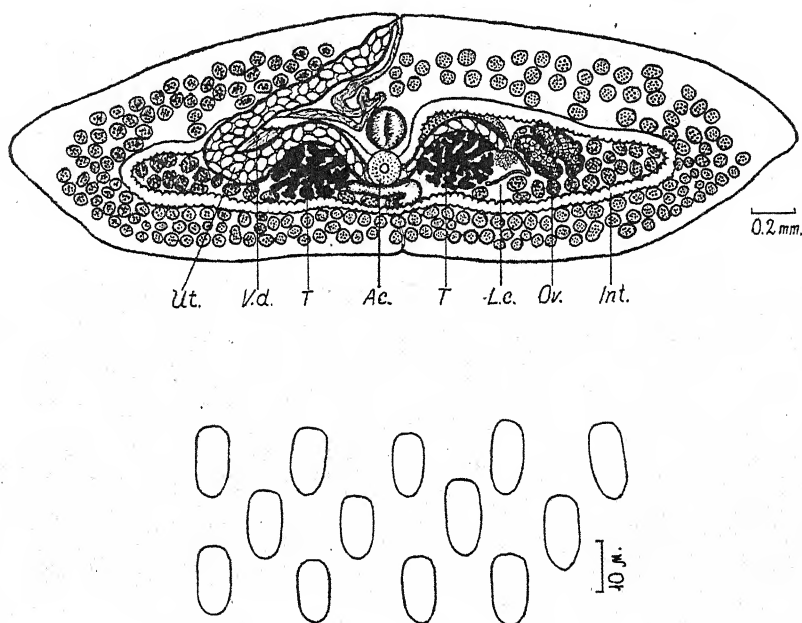


FIG. 1. (Top) Total view of *Transversotrema haasi*. Ac.—Acetabulum; Int.—Intestine; L.c.—Laurer's canal; Ov.—Ovary; T.—Testis; Ut.—Uterus; V.d.—Vas deferens. (Bottom) Body spines.

### Description

Body flat, transversely elongated, lancet-shaped, length 0.8–1.25 mm, width 2.5–4.0 mm; ventral surface slightly concave, dorsal one slightly convex, both covered with short, stout spines disposed quicunctially; the spines are slightly larger, 15  $\mu$ , on the ventral surface than on the dorsal one. The oral opening has the shape of a longitudinal slit in the center of the body, opens into a globular pharynx, 0.2 mm in diameter; there is no oral sucker projecting above the surface of the body. Immediately behind the oral opening there is a flat disc-shaped acetabulum (not stainable *in toto* but plainly visible on sections) covered with a few concentric rows of minute spines. The oral opening leads to a narrow esophagus which divides into two intestinal branches. The latter bend forward, then towards the lateral extremities of the body and, before reaching them, bend again towards the center to unite between the oral opening and the posterior ridge of the body. Thus the intestine forms a closed loop running almost parallel to the edges of the body. The course of the intestine is rather smooth, but the contractions of the wall produce an almost con-

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<sup>1</sup> In honour of Dr. G. Haas, lecturer in Zoology, who kindly presented the material.



tinuous row of small diverticula. Two testes lie at the sides of the acetabulum, inside the intestinal circle. They occupy a globular space, 0.3–0.4 mm in diameter, but their structure is dendroid. Vasa efferentia unite just to the right of the right testis to form a voluminous, coiled, vas deferens which gradually becomes narrower and opens into the genital sinus in the middle of the anterior ridge of the body. There is no copulatory organ. The ovary consists of 2–3 big irregular lobes: it is a little smaller than the testes and lies between the left testis and the intestinal bend. A short funnel-shaped oviduct connects it with the oötype where it meets the Laurer's canal, uterus and yolk duct. The oötype is surrounded by a Mehlis' gland complex. The uterus has a transverse and an ascending part. The transverse part passes over the testes and behind the acetabulum, thus forming two waves. The uterus is more or less distended by numerous eggs, depending on the age of the worm; it opens into a common genital sinus. The yolk glands consist of numerous follicles which occupy almost the whole space free from other organs inside and outside the intestinal loop. Their secretion accumulates in a yolk sac lying between the first bend of the uterus and the first bend of the intestine. There is no seminal receptacle, its function is taken by the proximal part of the uterus which contains a mass of spermatozoa. The ova are 90  $\mu$  long and 70  $\mu$  wide; they have no filaments and contain a formed miracidium. The excretory vesicle is transversely elongated. The right extremity receives the excretory tubes from both sides of the body and the left one, widened and bent towards the middle line of the body, joins a narrow excretory duct which opens to the surface in the middle of the posterior edge of the body, just opposite the genital sinus.

The above described trematode does not correspond to any known genus and it does not fit into any known family. It is an aberrant form in every respect. In its asymmetrical transverse structure it resembles only one known species, *Moreauia mirabilis* Johnston, with which it has no taxonomic relation. In the absence of a copulatory apparatus and seminal receptacle and in parasitizing fish it recalls the family MESOMETRIDAE Poche. However, the circular intestine, the absence of the oral sucker, the presence of the acetabulum and of a peculiar excretory duct make the relation to this family doubtful. A new subfamily TRANSVERSOTREMINAE is proposed for this genus.

## ON A NEW GENUS AND SPECIES OF PARASITID MITE

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### INTRODUCTION

In the course of experimental studies on the German cockroach, *Blattella germanica* (Linné), being carried on in this laboratory, 25 specimens of mites, representing a new species of the family *Parasitidae*, were found in cages housing the roach colony. As these specimens, 21 females and 4 males, differ from forms previously described, it seems advisable to erect a new genus and species for their proper classification. A new genus *Blattisocius* is proposed to include this mite, which, as *Blattisocius triodons*, is designated type species of the genus.

All specimens were killed in hot water, and mounted in a polyvinyl alcohol, lactic acid, phenol medium.

#### *Genus Blattisocius* gen. nov.

Color white. Movable chela bearing three teeth. Fixed chela about a third as long as movable chela, with a seta-like process near its tip. Anal plate bearing four paired and one unpaired setae, anteriorly truncate, larger than sternal plate. Peritremal and parapodal plates present. A pair of spindle-shaped plates present, one of which lies on either side of the dorsal plate between the latter and coxae I and II. A pair of large setae at posterior margin of dorsal shield.

#### *Blattisocius triodons* sp. nov.

*Female*: Color white, body longer than broad, shoulders not prominent. Legs II and III about equal in length. Legs I and IV about equal in length and longer than legs II and III. Tarsi of all legs bearing caruncles. Dorsal shield entire, not covering all of the dorsal surface. This shield bears about 32 pairs of setae, the most conspicuous being a very long pair at its posterior margin, and two pairs of small setae at its anterior margin (Fig. 1). Sternal plate rectangular with two lateral projections near its anterior end. Extends from anterior margin of coxae I to posterior margin of coxae II. Slightly longer than wide. Bears three pairs of setae. Genito-ventral plate flask-shaped, posteriorly truncate. Extends from anterior margin of coxa III past posterior margin of coxa IV. Bears one pair of spines. Anal plate larger than sternal plate. Slightly more than two-thirds as broad as long. Anterior margin straight. Bears four paired setae and one unpaired seta (Fig. 2). Peritremal and parapodal plates present. Spiracles between coxae III and IV (Fig. 3 and 4). Small spindle-shaped plate on either side of dorsal plate, lying between dorsal plate and coxae I and II. Each plate connected with a peritremal plate by a single very narrow chitinous strand (Fig. 3). Movable chela bearing three teeth, the most distal of which is largest. This chela is nearly straight throughout its length, slightly curved near tip, terminating in a point. Fixed chela about a third as long as movable chela, rod-like, a seta-like process near tip (Fig. 5). Palpi five-jointed and filariform. Labium with four pairs of setae. Chaetotaxy normal, dorsal and ventral surfaces of the opisthosoma possessing numerous setae. A pair of exceptionally large setae at the posterior margin of the dorsum.

#### Measurements of type specimen:

Total length (to anterior border of labium), 609 microns.

Body length (without capitulum), 504 microns.

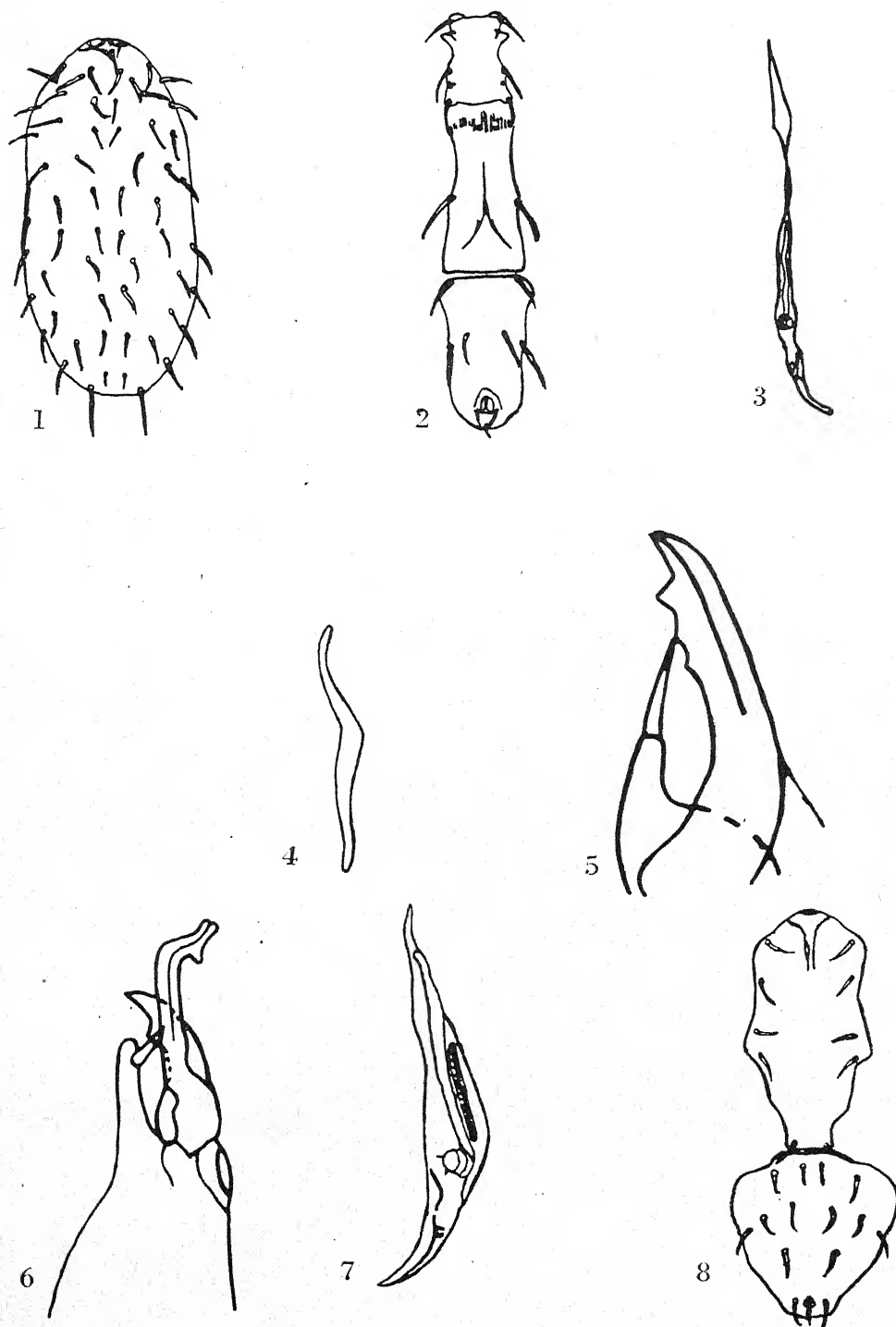
Body width, 301 microns.

Leg I, 441 microns; Leg II, 301 microns; Leg III, 315 microns; Leg IV, 420 microns.

*Male*: The male has the same general characteristics as the female. The mouthparts show sexual differentiation as in males of many species (Fig. 6). Peritremal plate differs from that of female (Fig. 7). Dorsal shield as in female. Ventral plates two in number. Anterior plate extending from anterior margin of coxa I to posterior margin of coxa IV. Posterior plate broad, roughly triangular (Fig. 8).

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- FIG. 1. Dorsal plate of female.  
 FIG. 2. Ventral plates of female.  
 FIG. 3. Peritremal plate of female, showing attached spindle-shaped plate, which lies dorsal to coxae I and II.  
 FIG. 4. Parapodal plate of female.  
 FIG. 5. Chelae of female.  
 FIG. 6. Chelae of male.  
 FIG. 7. Peritremal plate of male.  
 FIG. 8. Ventral plates of male.

## Measurements of type specimen:

Total length, 410 microns.

Body length, 385 microns.

Body width, 217 microns.

Leg I, 322 microns; Leg II, 294 microns; Leg III, 287 microns; Leg IV, 329 microns.

*Habitat*: Debris in colony of German cockroaches, *Blattella germanica* (Linné).*Cotypes*: Two females and one male deposited in the U. S. National Museum.

## DISCUSSION

Only three mites were found on 238 roaches examined with the aid of a dissecting microscope. All other specimens were obtained from debris taken from the floor of the roach cages.

## SUMMARY

*Blattisocius triodons*, representing a new genus and species of mite of the family Parasitidae, is described. A total of 25 specimens of this mite were taken from debris in cages housing a colony of German cockroaches, *Blattella germanica* (Linné). Of these specimens, 21 are females and four males. Only three mites were found on 238 roaches examined.

## ACKNOWLEDGMENT

The writer wishes to express his appreciation to Lt. T. F. Brunner, MAC, Mrs. Ruth Pégau, and Miss Hilda Jean May for technical assistance received during the course of this study.

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PERICARDITIS IN *XENOPUS LAEVIS* CAUSED BY *DIPLOSTOMU-  
LUM XENOPI* SP. NOV., A LARVAL STRIGEID

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INTRODUCTION

The metacercariae of the strigeid trematodes usually occur, either as non-encysted or encysted forms, in the tissues of aquatic animals, particularly fishes and amphibians. Considerable damage to the tissues is produced and these infections often cause death (see Van Haitsma, 1931; Nigrelli, 1943). In these secondary hosts, practically every organ of the body may be attacked. Thus strigeid larvae have been found invading the skin, muscle, eyes, mesenteries, liver, kidney, spleen, gonads, brain and heart. Adult worms develop to sexual maturity in the intestine of reptiles, birds and mammals. The mature parasites also are capable of eliciting tissue reactions in some of the definitive hosts, causing such lesions as enteritis or tissue hyperplasia.

The strigeid larvae which produce pericarditis in *Xenopus* were previously reported by Southwell and Kirschner (1937). The present contribution deals with the pathology resulting from this infection, and a redescription of the fluke, together with notes concerning its taxonomy and probable life-history.

The writers wish to acknowledge the helpful suggestions and criticisms of Professor H. W. Stunkard, New York University.

PATHOLOGICAL PHYSIOLOGY

The larval flukes are found within the pericardial sac of South African toads and with the aid of a hand lens can be seen moving about in the fluid under the transparent pericardium (Fig. 1). The number of flukes present, particularly in toads that were dying, varied from 25 to 150. Other apparently healthy *Xenopus* invariably showed two or more of these parasites. Of 55 toads examined during the past two years, 43 were found to be parasitized, and those that died during the summer months were always heavily parasitized.

The parasite constitutes a foreign body, hence the protective response of the serous pericardial membrane is that of exudation. In the initial stages, the amount of fluid produced is small and therefore there is little if any effect on the heart. However, the continuous activity of many parasites, along with the possible toxic effects of their waste products, promotes the production of greater amounts of fluid. As the pericardial sac distends, it naturally encroaches upon other vital thoracic organs, especially the lungs. It is clear, therefore, that the lungs can no longer expand to their normal limits, and the result is a diminished vital capacity.

When the pericardial sac can distend no further, the intra-pericardial pressure rises until it reaches a point where it is greater than the pressure in the superior and inferior venae cavae. At this point a vicious cycle begins. Undue pressure on the

sinus venosus, the superior and inferior venae cavae, and on the pre-caval veins interferes with the inflow of blood into the right auricle. This results in a diminished ventricular output with a corresponding drop in the systemic pressure, which in turn



FIG. 1. Dorsal view of the heart of *Xenopus laevis*. The strigoid flukes can be seen through the transparent pericardium. (Photograph by S. C. Dunton, N. Y. Zoological Society.)

results in slower oxygenation of the blood at the various respiratory organs, namely the lungs and skin. The anoxemia that results slowly paralyzes the respiratory centers and respiration ceases.

The clamping effect of abnormal pressure on the venae cavae, the sinus venosus, etc., tends to produce a congestion and subsequent stagnation of poorly oxygenated blood in the somatic tissues and viscera. This may account for the apparent vomiting reflex, which may be so powerful that an eversion and protrusion of the stomach may occur.

The efficiency of the heart is greatly diminished. The toads therefore die as a result of a true auto-toxemia resulting from a severe anoxemia. The affected heart

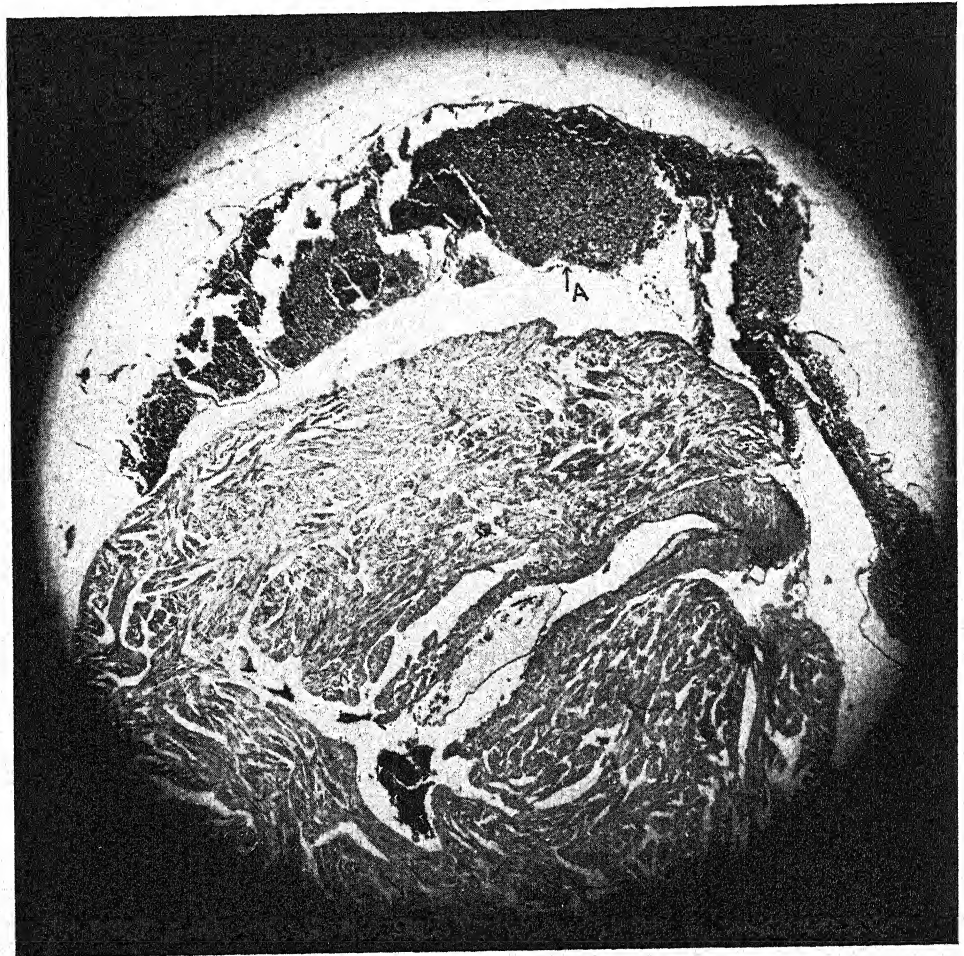


FIG. 2. Section through the ventricle of the heart, showing encapsulated semilunar haematoma (A) immediately below the epicardium. Intraventricular hemorrhages are evident within the myocardium, Haem.-Eosin. (Photograph by S. C. Dunton, N. Y. Zoological Society.)

continues to beat rhythmically after the respiratory centers no longer function, and finally it stops in systole, while the auricles continue to contract for some time.

Occasionally, as observed in several specimens, death is produced by haematoma-like formation within the ventricular wall (myocardium proper) or immediately under the epicardium (Fig. 2). Extravasation of blood into the pericardial sac, however, does not occur. This phenomenon may be explained by the fact that the fluid in the pericardial sac acts as a tampon on the bleeding points. In many



instances, extensive adhesions were present between the pericardium of the abnormally distended heart and the ventral thoracic wall.

#### DESCRIPTION OF THE PARASITE

The live strigeids of *Xenopus* are linguiform (Fig. 3). When examined under the microscope their movements are active, with the posterior end capable of expansion and contraction, much like the tail appendage in members of the Hemiuridae. However, this hindbody is not as well developed as in some other strigeids, although definitely demarcated from the forebody. When fully expanded, it is at least one-third the length of the forebody and cylindrical in form. If the hindbody is extended, the lateral margins of the forebody tend to fold ventrally. Such an infolding also occurs when the worms are freely suspended in the medium in which they are being observed. If the worms are in contact with the substratum, or if the hindbody is contracted, the flukes flatten out and the lateral folds disappear. In many of the fixed and stained specimens a slight infolding is found at the posterior lateral margins, particularly at the region where the fore and hindbodies meet.

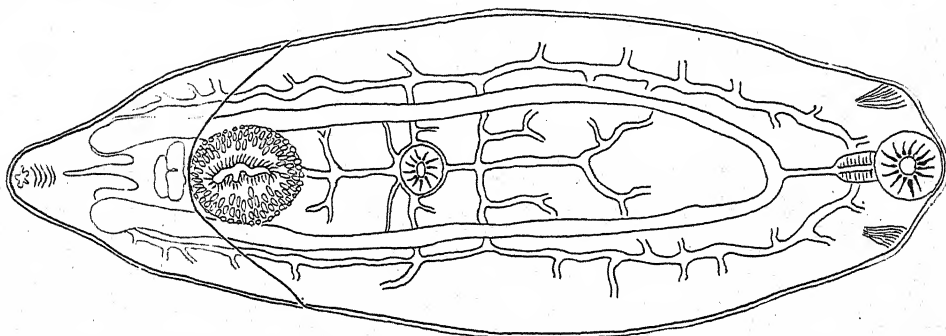


FIG. 3. Sketch of *Diplostomulum xenopi* made from a photomicrograph of a living worm. Details of tribocytic organ, cotylae, reserve excretory system, etc., added from observations on stained preparations. ( $\times 125$ .)

The anterior end of the strigeid shows a slight lateral or auricular projection on either side of the oral sucker and these structures are especially evident in larger worms. The oral sucker is subterminal, well developed and followed by the pharynx. The oesophagus is relatively long and the intestinal crura pass to the posterior end of the body, deflect around the holdfast organ, terminating inside the hindbody. The lateral suckers or cotylae are poorly developed but possess both muscle fibers and gland cells. The acetabulum is comparatively small and oval in shape. It is centrally located in the anterior part of the posterior half of the forebody, some distance in front of the tribocytic or holdfast organ. The latter structure is large, oval in shape, the long axis corresponding to the long axis of the body. The cavity of the tribocytic organ is bordered by papillae and numerous proteolytic gland cells cover the dorsal surface. The primordia of the gonads are present just behind the tribocytic organ but within the hindbody. The excretory pore which is stellate in shape is found at the posterior tip, with branches of the reserve excretory system ramifying throughout the body. The system is typical of the strigeids. The main branches pass posteriorly from the region of the pharynx, and at the level of the anterior border of the acetabulum receive the branches coming from the intercecal region between the acetabu-



lum and the point of bifurcation of the ceca and between the acetabulum and tribocytic organ, and those branches coming from the extracecal areas of the posterior and anterior parts of the body. The main vesicles dilate and unite immediately before they reach the excretory pore. The body of the worms is filled with many calcareous concretions normally associated with such a reserve excretory system.

Specimens were fixed in warm bichloride of mercury with acetic acid and stained with paracarmine or Delafield's hematoxylin. In these preparations most of the concretions are dissolved. The flukes usually take on a more or less oval and flattened shape. The size of the worms varies considerably and many of the characteristics described above are obscured. For matter of record the following measurements of fixed and stained worms are given: Size of the body varies from  $0.2 \times 0.25$  to  $0.76 \times 0.31$  mm (av.  $0.52 \times 0.3$  mm); oral sucker, 0.04 mm; pharynx,  $0.017 \times 0.008$  mm; acetabulum,  $0.038 \times 0.045$  mm; holdfast organ,  $0.11 \times 0.17$  mm; distance between acetabulum and tribocytic organ varies from 0.17 to 0.33 mm. In these stained specimens, the oesophagus, intestinal crura, reserve excretory branches and limits of the hindbody become indistinct for detailed study. The cuticle is thin and without spines. It should be pointed out that the description and measurements given here differ on many points from those reported originally by Southwell and Kirschner (1937).

#### CLASSIFICATION OF STRIGEIDS FROM *Xenopus*

The strigeids of the clawed toad are definitely diplostomes. They were found in the pericardial sac immediately on arrival of the anurans in this country from South Africa, and they were continuously recovered from toads that died during a period of more than two years after their importation.<sup>1</sup> This indicates that the flukes never encyst and the infection may persist for as long as the toads remain alive. The fact that worms of varying sizes were found in the pericardial fluid demonstrates that the toads were being continually reinfected while in their native habitat. Examination of other organs and tissues of the body revealed no parasites or evidence which would indicate the migration path taken by these flukes, and further shows that by the time the toads arrived in this country the worms had already reached their final location in the host.

Other strigeids have been reported from the heart region of various hosts. Many species of the group Tetracotyle are invariably found in thick cysts in the pericardium of marine and fresh-water fishes (see Van Cleave and Mueller, 1934; Nigrelli, 1943). Odlaug (1940) reported the mesocercariae of *Alaria intermedia* as occurring free, among other tissues and organs, in the pericardial region of tadpoles, and frogs (*Rana pipiens*) less than a year old. The metacercariae and adults of *A. intermedia* are found in mammals. A similar four-host cycle was reported by Bosma (1934) for *Alaria mustelae* in which the mesocercarial forms are found in the tissues of frogs (*Rana catesbiana* and *R. clamitans*) and metacercariae in mammals.

The presence of lateral suckers and primordia of the gonads shows that the strigeids of the clawed toad are metacercarial and not mesocercarial forms. The fact that these worms are easily digested at room (ca. 25° C) and incubator temperatures

<sup>1</sup> These anurans are imported for use in the biological assay of sex hormones and determination of human pregnancy (See Weisman, Snyder and Coates, 1942).

(37° C) when exposed to artificial ferments<sup>2</sup> would suggest that final host may be some reptilian form.

In 1936, Dubois erected the family PROTERODIPLOSTOMIDAE to include the strigeids parasitic in reptiles. The family is a fairly homogeneous group and is characterized mainly by the position and arrangement of the reproductive systems and sub-divided on the basis of size and shape of the tribocytic organ, the presence or absence of papillae on this organ and differences in the distribution of the vitellaria. With the exception of *Proalaroides serpentis* Yamaguti (1933), the forebody of the flukes are more or less flattened and all lack the lateral suckers. There are striking similarities between the strigeids of *Xenopus* and the members of the PROTERODIPLOSTOMIDAE Dubois, 1936. Except for the presence of the lateral suckers, the worms are closely similar to certain POLYCOTYLINAE Monticelli, 1888, such as *Crocodilicola* Poche, 1925, and *Pseudocrocodilicola* Byrd and Reiber (1942). It is with difficulty that the lateral suckers of the strigeids of the clawed toad can be demonstrated but there can be no doubt that the cotylae are present. The question arises, because of this fact, whether or not these lateral suckers have been overlooked by other investigators or whether or not they disappear as the worms reach maturity. It is unfortunate that the life-history is not known for the typical members of the family. But it may be suspected that the larval forms show up in amphibians. This is known to be the case for *Proalaroides serpentis* Yamaguti, a proterodiplostomid (OPHIODIPLOSTOMINAE Dubois, 1936) which occurs in the intestine of the cobra, *Elaphe quadri-virgata*, and the metacercariae of which are found encysted in the musculature of the frog, *Rana nigromaculata*. *Proalaroides serpentis* has well developed cotylae, but the size and shape of the body and the tribocytic organ together with other organ relationships eliminates this genus as the probable adult of the strigeids of *Xenopus*.

Southwell and Kirschner (1937) concluded that the strigeids from the clawed toad were identical with *Tylodelphys rachiaea* (Henle, 1833), a larval form redescribed by Lühe (1909) from the rachidian canal of the European frog (*Rana temporaria*). Lühe suggested that the adult form may be *Hemistomum excavatum* (Rud., 1803). This assumption was shown to be correct when Ciurea (1928) experimentally demonstrated that the larval *T. rachiaea* from frogs developed into *Proalaria excavata* (Rud.) in the small intestine of the stork, *Ciconia ciconia*. This part of the cycle was confirmed by Szidat (1935) and the life history completed by his discovery of the forked-tailed cercarial stage in *Planorbis corneus* (L.). It should be pointed out that *Tylodelphys rachiaea* (larval forms), *Hemistomum excavatum* and *Proalaria excavata* are considered by Dubois (1938) to be synonyms of *Tylodelphys excavata* (Rud.). All the described species of *Tylodelphys* occur in birds, and the metacercariae are found in fishes or amphibians.

The chief characteristics of the genus *Tylodelphys* are: a distinct trilobed appearance of the anterior end, weakly developed but nevertheless distinct lateral suckers, presence of a prepharynx, a relatively short esophagus and comparatively large tribocytic organ. The strigeids from the African clawed toad have weakly developed but indistinct lateral suckers, no prepharynx, relatively long esophagus and medium sized tribocytic organ possessing papillae. Such characteristics certainly eliminate these strigeids from the genus *Tylodelphys*. However, on the basis of distinct differ-

<sup>2</sup> 1 gr. of pepsin in 100 cc of N/0.01 HCl; 50-50 mixture of 1% pancreatin and 0.5% sodium bicarbonate.

ences in organ relationships, size, shape and structure of the tribocytic organ, distribution of the proteolytic gland cells, degree of development of the lateral suckers, site of infestation, differences in host and geographical location of the host, we consider the strigeids from *Xenopus* to be different from those previously described from other amphibians and for these reasons they are tentatively identified as *Diplostomulum xenopi*, sp. nov.

## SUMMARY

The pathological physiology of the heart of *Xenopus laevis* resulting from strigeid fluke infection is described.

The parasite producing the pericarditis and other lesions of the heart is redescribed and considered to be a new species for which the name *Diplostomulum xenopi* is proposed.

The taxonomic relationships are discussed and probable life-history suggested.

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CERCARIA SOLEMYAE N. SP., PROBABLY A BLOOD FLUKE, FROM  
THE MARINE PELECYPOD, SOLEMYA VELUM

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In the summer of 1937, during the course of a survey of trematode infections in molluscs, some cercariae were obtained from the bivalve, *Solemya velum*, collected in the vicinity of Woods Hole, Massachusetts. During subsequent summers, similarly infected *Solemya velum* have been collected but never in great numbers. The anatomy of these cercariae strongly suggests a close relationship to the blood-parasitizing trematodes.

MATERIAL AND METHODS

Specimens of *Solemya velum* were placed in finger bowls of sea water and observed daily for the emergence of cercariae. Live cercariae, fixed and stained whole mounts, and serial sections, were studied.

SPOROCYST

(Fig. 6.)

*Cercaria solemyae* n. sp., develops in small, oval or spherical sporocysts in the gonad and digestive gland of the bivalve mollusc, *Solemya velum*. Some infections are extremely heavy and must result in disturbances of digestion and reproduction of the host. Fig. 7 shows a severe infection. A daughter sporocyst generation is present. Few cercariae are contained in a sporocyst, not more than five were observed in any one. The wall of the sporocyst is thin except at one end where a knob of cells occurs.

The incidence of infection seems to vary with different collecting grounds. From some regions no infected individuals could be found. Sixty-three *Solemya velum* were collected from one locality and all were negative. From other localities the infection rate was as high as 9 per cent.

CERCARIA

(Figs. 1-5)

The body of the cercaria is small, measuring (living material, three specimens) from 0.089 mm to 0.098 mm, averaging 0.094 mm in length by 0.025 mm to 0.030 mm, averaging 0.027 mm in width. Measurements of fixed material (ten specimens) range from 0.059 mm to 0.078 mm with an average of 0.067 mm in body length by 0.022 mm to 0.034 mm with an average of 0.026 mm in body width. The sides of the body present a crenated appearance partly due to short rows of relatively large spines. There are about twenty-eight rows of these spines on each side of the body, each row including three or four spines. These spines are about three microns in length. In addition to these, there are longer, bristle-like spines on each side of the body, some near the exits of the cephalic gland ducts and others located a distance of about one-fifth the body length from the posterior end. No suckers are present. The mouth is subterminal and has an irregular outline which varies somewhat as shown in Figs. 2 and 3. The digestive tract consists of a slender tube leading from the mouth to the pouched gut near the middle of the body. The gut has five pouches in most specimens but occasionally only four. The cephalic glands are five in number on each side of the body. The gland cells are somewhat laterally arranged and occupy the middle two-fourths of the body. They stain deeply and quickly with

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neutral red. Their ducts pass antieriad in a group and empty into two pit-like depressions at the anterior end of the body (Fig. 4). The distal ends of the ducts may be swollen and are arranged in a circle so that in cross section they form a rosette (see Fig. 5). The excretory system has been partially traced. The excretory bladder is divided into three parts, two small, lateral sacculations and a middle larger one. The entire bladder measures about 0.0036 mm long by 0.0058 mm wide. The lower ends of the main collecting ducts were seen but could not be traced cephalad. Two minute flame cells were seen on each side of the body but it is highly probable that there are more.

The tail is very short and is capable of only limited change in size and shape from almost spherical to pyriform. Tail measurements of living material ranged from 0.016 mm to 0.019 mm with an average of 0.017 mm in length by from 0.011 mm to 0.014 mm with an average of 0.0125 mm in width. The surface of the tail is crenated like the body. There are two bristles that project outward, one from each side of the tail. An excretory tube extends from the bladder into the tail where it divides into two blind sacs. The tail has practically no function as an organ of locomotion. In fact, the cercaria as a whole moves very little.

*Cercaria solemyae* is most like the cercariae and adults of the family APOROCOTYLIDAE. It resembles these forms in the absence of suckers and in the possession of groups of spines laterally arranged [similar to *Sanguinicola armata* and *S. intermedia*, Ejsmont (1925), and *Paradeontacylix sanguinicoloides* McIntosh (1934)]; a gut provided with pouches [similar to the cercaria of *Sanguinicola* Odhner (1911), adult of *Paradeontacylix sanguinicoloides* McIntosh (1934), and adult of *Deontacylix ovalis* Linton (1910)]; and an irregular mouth outline [similar to *Sanguinicola armata* Ejsmont (1925)]. It differs from all known blood-fluke cercariae in the nature of the tail, since all the others have forked tails.

There are, of course, pitfalls in anatomical similarities but several characters of *Cercaria solemyae* are so strikingly similar to certain blood fluke cercariae or adults, particularly those parasitizing fishes, that it is believed that this form is a new type of blood fluke cercaria. Some attempt has been made to complete the life cycle but due to lack of time it was not successful. It is hoped that after the war, this work may be carried on to a satisfactory conclusion.

#### EXPLANATION OF PLATES

##### ABBREVIATIONS USED

C—cephalic gland	G—germ ball
Ce—cercaria	I—intestine
D—duct of cephalic gland	M—mouth
E—excretory bladder	T—excretory tube in tail
F—flame cell	

#### PLATE I

FIG. 1. Camera lucida drawing of *Cercaria solemyae* n. sp.

FIGS. 2 and 3. Outlines of the shape of the mouth.

FIG. 4. One of the pits at the anterior end of the cercaria into which the cephalic glands empty.

FIG. 5. Cross section of the swollen portions of the cephalic gland ducts showing their rosette arrangement.

FIG. 6. A sporocyst containing two cercariae and a germ ball.

#### PLATE II

FIG. 7. Longitudinal section through the body of the clam, *Solemya velum*, showing the parasitized gonad (1), digestive organ (2) and a ganglion of the host (3).

FIG. 8. Section adjacent to the one shown in FIG. 7, but under higher magnification. Below the host ganglion (3) are seen parasitized gonad tubes.

FIG. 9. Some of the parasitized gonad tubes under still higher magnification (4) indicates a cercaria within a sporocyst.

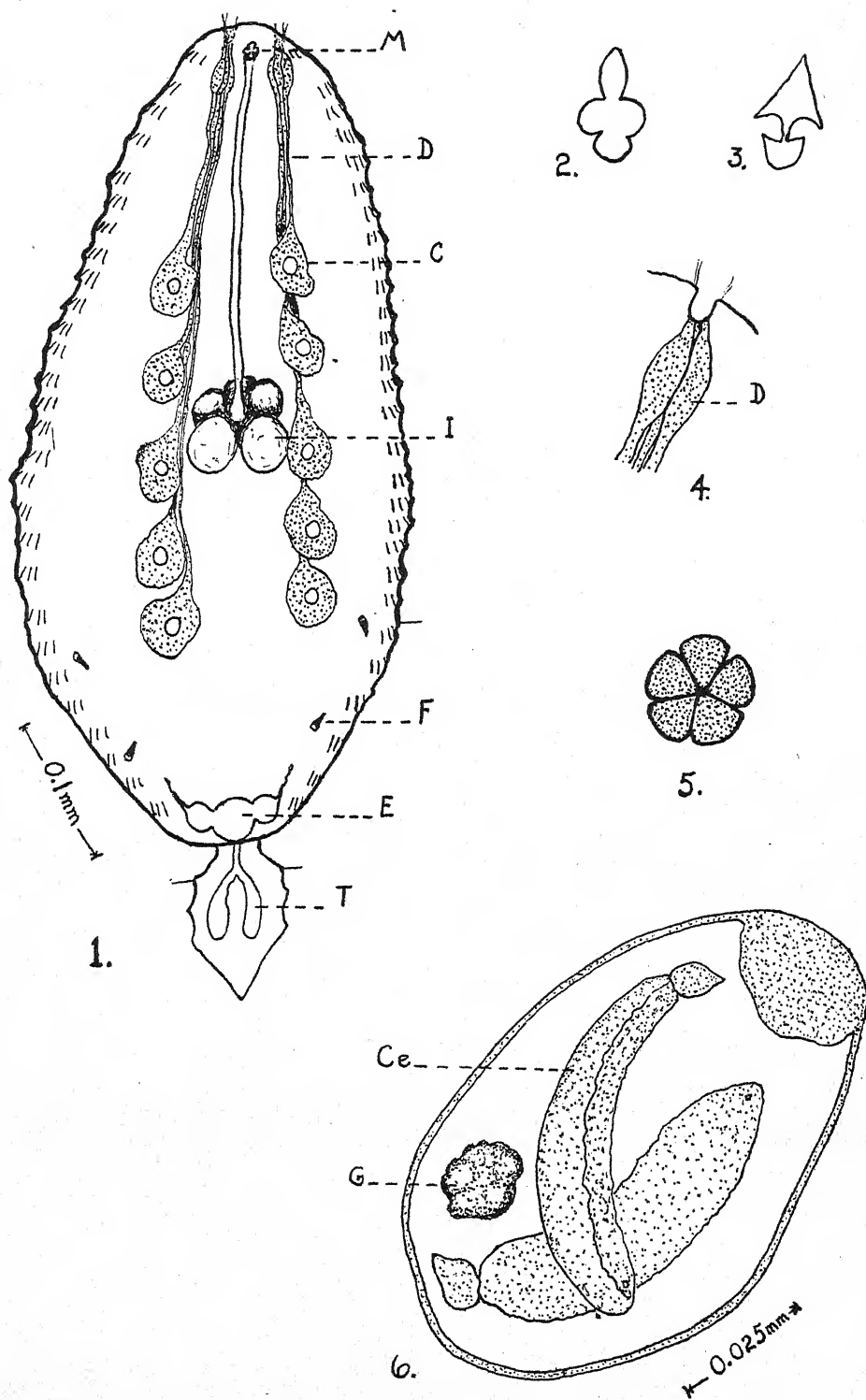


PLATE I

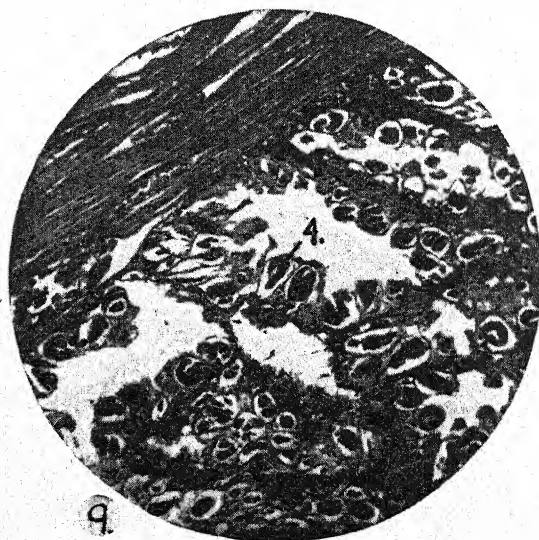
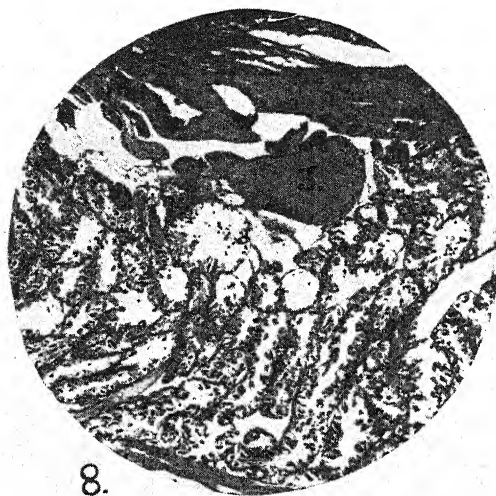
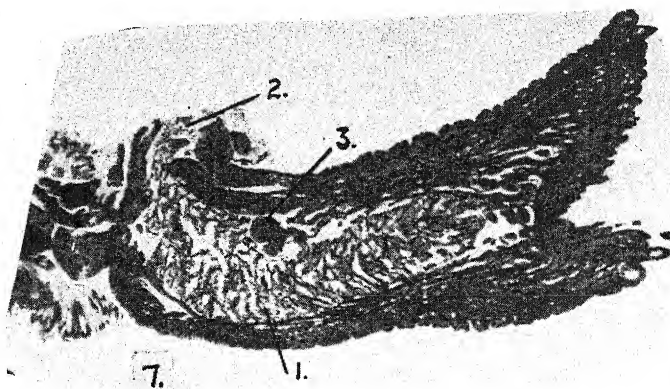


PLATE II

## SUMMARY

A new species of larval trematode, *Cercaria solemya*, developing in sporocysts in the pelecypod, *Solemya velum*, is described.

The anatomy of the body of this cercaria strongly suggests that it is one of the blood flukes.

The tail of this cercaria is very small and is not forked, thus differing from all known blood fluke cercariae.

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## WAR-TIME ACTIVITIES OF MEMBERS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

HENRY E. MELENEY\*

In my presidential address for 1942,<sup>1</sup> I outlined some of the ways in which members of the American Society of Parasitologists might contribute to the winning of the war. In December 1943 it was decided to determine, as far as possible, the current war-time activities of the members of the Society. Dr. James T. Culbertson, Secretary of the Society, sent return postcards to the 426 members in good standing asking for information with reference to their present employment and activities. After most of these cards were returned, it was decided to send a questionnaire to the 101 members who were delinquent in their dues, in view of the fact that some of them were undoubtedly located where they could not keep up their active membership.

As a result of this survey, information concerning 442 (84 per cent) of the members of the Society has been received and is presented in Table 1. It will be noted that 80 members of the Society were in military service, 36 of these being already overseas when the cards were sent in. It is probable that a considerable number of the members from whom replies were not received are also in military service overseas and did not have an opportunity to reply. Accurate data would probably indicate that about 20 per cent of the membership is in military service.

Among the 69 members employed by civilian governmental services the large majority are undoubtedly occupied in research or administrative positions involving war activity. Two of these are known to be in overseas service.

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<sup>1</sup> Meleney, H. E. 1943. The Role of Parasitologists in World War II. Am. J. Parasitol. 29: 1-7.



TABLE 1.—*War-time activities of 442 members of the American Society of Parasitologists as of December 1943–April 1944*

Military Service, 80.		Private Research Institutes .....	3
Army, 62.		Rockefeller Foundation .....	9
Continental United States .....	33	Medical Students .....	10
Overseas .....	29	Commercial Firms, 9.	
Navy, 18.		Pharmaceutical .....	4
Continental United States .....	11	Other .....	5
Overseas .....	7	Hospitals and Medical Laboratories .....	7
Civilian Governmental Services, 69.		Puerto Rican Members .....	4
U. S. Public Health Service, 13.		Retired .....	9
Continental United States .....	12	Miscellaneous .....	8
Overseas .....	1	Foreign Members in Allied Countries .....	34
U. S. Department of Agriculture, 28.		Members in Enemy Occupied Territory, 15.	
Bureau of Animal Industry .....	17	Philippines .....	5
Bureau of Entomology .....	7	China .....	4
Other .....	4	Japan .....	3
Other Federal Agencies, 8.		Germany .....	2
Continental United States .....	7	Belgium .....	1
Overseas .....	1		
State Governmental Agencies .....	17	Total .....	442
Local Governmental Agencies .....	3		
Civilian Teaching Positions, 185.			
Universities, Colleges, High Schools .....	117		
Medical, Dental, Veterinary, Public Health Schools .....	68		

The largest group of members, 185, is engaged in teaching and research in civilian educational institutions. More than one-third of these are in schools related to medicine and are, therefore, directly occupied in teaching students who are already in military service. Undoubtedly, many of those in other universities and colleges are teaching students who are in military service. In addition, a considerable number in both medical and non-medical institutions are engaged in government research projects or in research directly or indirectly connected with the war effort.

Among the other groups, it will be noted that there are 10 medical students, and that institutions or agencies are represented which are devoting their major effort to war activities.

The Puerto Rican members are listed separately to indicate that they are not in the continental United States, although they are not to be included among the foreign members in allied countries.

Finally, particular attention should be called to the 16 members of the Society in enemy occupied territory. Those in the Philippines and China consist of natives of those countries who are under the domination of the Japanese. The single member in Belgium is also under enemy domination. The three members in Japan and the two in Germany are natives of those countries.

This brief summary indicates that the American Society of Parasitologists is taking an important part in the war effort. It is probable that before the war is over more of the members will enter military service and others will undoubtedly undertake research projects under government auspices. The tremendously increased attention which is being given to human parasitology as a result of the war will undoubtedly create a permanent interest in this field which will be reflected in a greater demand for the teaching of this subject in the curriculum of medical schools and for the solution of research problems.

## RESEARCH NOTES

### THE EFFECT OF PRICKLY-ASH BARK UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS

Phenothiazine has proved itself an anthelmintic of unusual merit although it possesses certain prominent disadvantages. For example the dose for some animals is bulky, the drug is somewhat expensive, and it may be toxic when used in some animals. An effective synergist might obviate in part some of these objections. The present report is an unsuccessful attempt to discover such a synergist, and it is recorded largely to direct attention to an unexplored field of phenothiazine research.

Haller, La Forge, and Sullivan (1942, J. Econ. Entomol. 35: 247-248) reported that sesamin and sesame oil, as well as asarinin which is found in the bark of southern prickly ash, activate the insecticidal properties of pyrethrum. Since the pure chemicals are not available some bark of southern prickly ash, *Xanthoxylum Clavo-Herculis*, was obtained. One gram of bark plus 0.2 gram of phenothiazine was given to each of 6 Ancona cockerels. Six similar cockerels, receiving 0.2 grams of phenothiazine alone, served as controls. Results are presented in Table 1. The

TABLE 1.—Comparative efficacy of 0.2 grams of phenothiazine and of 0.2 grams of phenothiazine mixed with 1 gram of *Xanthoxylum Clavo-Herculis* bark for the removal of *Ascaridia* and *Heterakis* from chickens\*

Band No.	Weight at time of treatment	Worms removed by treatment		Worms found at necropsy		Per cent	
		Ascaridia	Heterakis	Ascaridia	Heterakis	Ascaridia	Heterakis
Phenothiazine mixed with bark of <i>Xanthoxylum Clavo-Herculis</i>							
	<i>Grams</i>						
3596	1715	0	5	8	0	0.0	100.0
3592	1505	0	129	32	1	0.0	99.2
3591	1560	0	88	59	0	0.0	100.0
3595	1860	0	25	1	11	0.0	69.4
3958	1540	10	16	25	0	28.6	100.0
3597	1600	0	6	1	0	0.0	100.0
Total		10	269	126	12		
Phenothiazine alone							
3662	1560	0	80	6	0	0.0	100.0
3663	1530	0	10	6	0	0.0	100.0
3671	2070	0	17	5	1	0.0	94.4
3665	1780	46	147	102	252	31.1	36.8
3666	1515	0	71	0	0	...	100.0
3667	2000	23	67	9	0	71.9	100.0
Total		69	392	128	253		

\* These Ancona cockerels were dosed January 10, 1943, and necropsied 3 days later.

mixture of phenothiazine with bark of *X. Clavo-Herculis*, removed 7.3 per cent of 136 *Ascaridia* and 95.7 per cent of 281 *Heterakis*; the phenothiazine alone removed 35 per cent of 197 *Ascaridia* and 60.7 per cent of 645 *Heterakis*. The total figures alone suggest that bark of *X. Clavo-Herculis* may have interfered with the activity of phenothiazine against *Ascaridia* and stimulated activity against *Heterakis*. However, the records of the individual birds suggest that the addition of bark was without effect since the differences in the percentages of worms removed are largely due in each case to the record of a very few heavily infected birds. We conclude, therefore, that 1 gram of the bark of *Xanthoxylum Clavo-Herculis* did not influence appreciably the efficacy of a dose of phenothiazine administered simultaneously.—PAUL D. HARWOOD AND JAMES E. GUTHRIE, Dr. Hess and Clark, Inc., Ashland, Ohio.

### NOTES ON THE FEEDING OF CONE-NOSED BUGS (HEMIPTERA, REDUVIIDAE)

Osimani (1942, J. Parasitol. 28: 147-154) studying some parasites of reptiles in South America fed *Triatoma rubrovaria*, a blood-sucking bug, upon the lizard, *Tupinambis teguixin*, after noting that Talice found these bugs in the burrows of this lizard. For the past few years, although many adults of *Triatoma longipes* have been collected at the Alvarado Mine in Arizona, a careful search of wood rat houses did not reveal this site as a breeding ground for this species of bug (Wood, 1941, Southwestern Med., April, 112-114). Possibly the nymphs of this Reduviid vector of Chagas' disease in the United States feed on reptiles in underground burrows as indicated by the following notes.

*Triatoma longipes* Barber. On May 18, 1942, during a period of relatively warm weather, the writer observed 3 small nymphs attempting to feed on the tail (1) and leg (2) of one western gecko, *Coleonyx variegatus*. Twenty-four hours later 4 of 20 small nymphs had fed to capacity in a jar with 5 adult geckos. On the same day, 26 starved small nymphs were put into a 10-inch Petri dish with 2 western worm snakes, *Leptotyphlops humilis humilis*. On May 20, 1942, these bugs were removed. One nymph was dead and 5 small nymphs fed on the snakes, two to capacity as evidenced by their well-rounded, dark-red abdomens. The remaining 20 apparently did not feed, since their abdomens were still flat in cross-section. These nymphs were placed in a jar with 1 adult ♂ desert scaly lizard, *Sceloporus magister magister*. After a 24-hour exposure, 4 were dead, 12 had been eaten by the lizard, and one of the remaining 4 live nymphs had fed to capacity on the lizard.

On May 20, 1942, a very warm day, 4 of 10 small *longipes* nymphs placed in a jar over a 24-hour period with 1 immature desert crested lizard, *Dipsosaurus dorsalis dorsalis*, fed on this lizard, some of them feeding to capacity. On the same day, 4 of 5 small nymphs fed on 1 adult western collared lizard, *Crotaphytus collaris baileyi*; 3 of 5 fed on 1 adult desert side-blotch lizard, *Uta stansburiana stejnegeri*; and 5 of 5 fed (three to capacity) on 1 desert leaf-nosed snake, *Phyllorhynchus decurtatus perkinsi*, each group over a 24-hour period. On the same day, 5 of 10 small nymphs fed (3 to capacity) on 2 immature yucca night lizards, *Xantusia vigilis*, resulting in the death of 1 lizard. Ten small nymphs placed in a jar with 2 adult western geckos, *Coleonyx variegatus*, were eaten by the lizards over a 24-hour period but the presence of blood spots on the sides of the jar (from crushed bugs) indicated that some of the bugs had fed on the geckos before being eaten.

On May 21, 1942, 35 small *longipes* nymphs, 18 of which were starved, i.e., with flat abdomens, were placed in a cage with 7 adult yucca night lizards, *Xantusia vigilis*. Nine of these had fed on the lizards by May 24th. On May 22, 1942, 2 of 11 small nymphs fed to capacity on 1 adult southern California alligator lizard, *Gerrhonotus multi-carinatus webbi*; 8 of 10 fed on 1 adult western glossy snake, *Arizona elegans occidentalis*; and 9 of 10 fed (6 to capacity) on 1 adult red racer, *Coluber flagellum frenatum*, each group feeding over a 48-hour period.

On June 23, 1942, 3 small nymphs of *Triatoma longipes* were placed in a jar at 3:00 PM with 1 Jerusalem cricket, *Stenopelmatus longispina* Brunner. By 3:50 PM, 1 nymph had fed on the cricket and 1 nymph was eaten by it. The *Triatoma* fed through the conjunctival membrane of the lateral abdominal wall. By 8:00 AM of June 24th, both of the remaining bugs were eaten by the cricket.

Thus, it appears that *Triatoma longipes* is not entirely dependent upon Arizona warm-blooded animals, especially mammals, for its food supply but may feed on reptiles and other cold-blooded vertebrates as Brumpt (1927, Presse Méd. 35: 1161-1162) reports for *Triatoma rubrovaria* from Uruguay.

*Triatoma rubida* (Uhler). On May 21, 1942, 3 of 5 small nymphs fed (2 to capacity) on 1 adult ♂ desert scaly lizard; 3 fed to capacity on 1 adult ♂ desert side-blotch lizard; 4 fed (2 to capacity) on 1 adult western collared lizard; and 2 fed on 1 desert leaf-nosed snake, each group within a 24-hour period. On May 25, 1942, 3 medium nymphs fed (2 to capacity) on 1 adult western gecko. On May 26, 1942, 2 ♂ *rubida* failed to feed on 1 western glossy snake, and 2 other ♂'s did not feed on 1 red racer, each over a 24-hour period.

*Triatoma protracta* (Uhler). On May 24, 1942, 3 medium nymphs fed (2 to capacity) on 1 adult ♀ desert side-blotch lizard; 1 did not feed on 1 ♂ desert scaly lizard; 1 fed to capacity on 1 immature desert crested lizard; 4 of 5 fed to capacity on 1 adult western collared lizard; 5 fed (3 to capacity) on 1 adult western glossy snake; and 5 fed (4 to capacity) on 1 adult red racer, each within a 24-hour period. Two medium nymphs were observed to feed immediately on 1 adult southern California alligator lizard.

The site of feeding when noted was the fine-scaled portion of the lizard's body, as side of neck, armpit, inguinal area or under body surface. Bugs feeding on the coarse-scaled portion of the lizard's body would apparently feed through the skin between the larger scales. Whether *Triatoma protracta*, *T. rubida*, and *T. longipes* feed in nature on cold-blooded vertebrates can be determined by examining the stomach fluids of recently captured bugs for nucleated red cells of characteristic size, shape and structure.—SHERWIN F. WOOD, 1015 No. Alexandria Ave., Hollywood 27, California.

#### PARAGLAUCOMA SP., A FACULTATIVE PARASITE OF COELENTERATES

Several specimens of *Hydra americana* collected from a pond on the University of Pennsylvania campus, examined during the spring of 1943, contained within their gastro-vascular cavities numerous active ciliates meeting in all details the morphologic criteria of the genus *Para-*

*glaucoma*. According to Kudo (1939, Protozoology, p. 550) this genus comprises only one representative species, *P. rostrata* Kahl, living in fresh water and often found in dead rotifers. Kahl (1926, Arch. f. Protistenk, 55: 351-353) reports finding them in a patch of moist moss and free living.

The organism present in *Hydra* did no serious damage, although on several occasions the ciliates were seen to escape from the digestive cavity to the external medium by forcing their way through the body wall of the host. The question of infection was somewhat complicated by the observation that the same ciliate occurred in the bodies of *Daphnia* which served as food for the coelenterates. They were, however, not seen in living *Daphnia*, so that the involvement of two or more hosts belonging to different phyla is entirely a matter of speculation. If *Paraglaucoma* is normally a scavenger, subsisting on decaying animal matter in fresh water, its complete immunity to *Hydra* digestive enzymes strongly suggests facultative parasitism. The ciliate was never encountered in *Chlorohydra viridissima*. The latter species was collected from the same source and kept in the same culture dishes with the infected *Hydra americana*.

During the summer of 1943 an organism strongly resembling the above *Paraglaucoma* was frequently found on and in living colonies of *Campanularia flexuosa*, gathered from the Provincetown breakwater. This parasite definitely did not enter the hydroid colonies with the food (via a primary host), but actively penetrated into mature gonangia or into injured portions of the hydrocaulus, where the protective perisarc was lacking. It never entered any hydranths through the manubrium, as it was not immune to the action of nematocysts. Aging hydranths which had lost their tentacles were attacked by the ciliate. Planulae of *Campanularia* kept for observation in Syracuse watch glasses were likewise attacked and destroyed. The parasite showed considerable size differences (60-110  $\mu$  long) and variation in opaqueness depending on the amount of food ingested.

Substances, probably enzymatic, secreted by the parasite had a remarkable cytolysing effect on the hydroid coenosarc. Within one hour this ciliate cytolyses from 30 to 40 times its own volume of host tissue, which demonstrates its potentially devastating inroads upon hydroid communities.

The aim of this note is not to suggest the establishment of a new species for the above two organisms, but to draw attention to the wide range of habitat (moss, pond water, hydra, marine hydroids, dead rotifers and dead daphnia) and the versatility of food habits of representatives of the genus *Paraglaucoma*. In its ecology *Paraglaucoma*, therefore, resembles *Glaucoma* which has been reported from an astonishing variety of potential hosts (1941, G. N. Calkins and F. M. Summers, Protozoa in Biological Research, New York, article by H. Kirby, Jr., pp. 894-899).—T. S. HAUSCHKA AND R. B. DOLL, University of Pennsylvania and Marine Experimental Station of the Lanckenau Hospital Research Institute, North Truro, Mass.

#### AN ADDITIONAL CALIFORNIA LOCALITY FOR *TRYPANOSOMA CRUZI* CHAGAS IN THE WESTERN CONE-NOSED BUG, *TRIATOMA* *PROTRACTA* (UHLER)

The writer, with the aid of members of United States Navy Malaria and Epidemic Control Team #40, collected 188 *Triatoma protracta* from 61 houses of the San Diego wood rat, *Neotoma fuscipes macrotis*, from November 4 to December 17, 1943, an average of three bugs per wood rat house. All the insects were taken 5 miles southwest of Fallbrook, San Diego Co., California. Twenty-seven of the 57 bugs naturally infected with *Trypanosoma cruzi* were collected within 150 yards of a large one-story building. The characteristic morphology of stained trypanosomes verified the diagnosis made from inspection of feces for living organisms in the 186 bugs examined. No *Triatoma* were found in the buildings.

The cone-nosed bugs examined included 20 ♂, 31 ♀, 43 large, 25 medium and 67 small nymphs, indicating a tendency for the occurrence in individual wood rat houses of either adults and large nymphs, or small nymphs at that time of year. The 57 (30%) naturally infected bugs included 8 ♂, 9 ♀, 22 large, 11 medium, and 7 small nymphs. The presence of *Trypanosoma cruzi* in the feces of small nymphs would indicate recent infection from the mammal host.

No mammals were trapped, but one ♂ southern parasitic mouse, *Peromyscus californicus insignis*, was caught by hand. Two uninfected bugs, one ♀ and one large nymph, fed on this mouse on November 11, 1943, were negative for trypanosomes when examined 35 days later. Fresh blood examination of the mouse was negative for trypanosomes.

One wood rat house from which 29 *Triatoma protracta* were collected contained 27 infected bugs (3 ♂, 4 ♀, 9 large, 5 medium and 6 small nymphs). The two negative bugs were small nymphs.—LT. (J. G.) SHERWIN F. WOOD, H-V(S), U.S.N.R. Forwarding Address: 1015 N. Alexandria, Los Angeles 27, Calif.



VIABILITY OF *TRYPANOSOMA CRUZI* IN CITRATED BLOOD STORED AT ROOM TEMPERATURE

In the investigation of possible cases of Chagas' disease (American trypanosomiasis) in Texas, blood specimens are collected at various localities and shipped to the central laboratory. In some instances this necessitates a delay of several days before the specimens can be examined. The question arose as to the suitability of such specimens for diagnosis, for it was not known how long *Trypanosoma cruzi* remained viable in citrated, whole blood stored at room temperature.

Material for such a determination consisted of citrated guinea-pig blood parasitized with *T. cruzi*. The animal was inoculated with a culture of the trypanosome on June 9, 1943. Examinations of wet-mount blood preparations on June 14th, 21st, and 26th, revealed no trypanosomes, but on June 28th, nineteen days after inoculation, approximately one trypanosome per low-power field was observed. Cardiac blood was citrated and stored in a sterile, cork-stoppered culture tube at room temperature. Whole blood was tested for *T. cruzi* complement-fixing antibodies by Davis' modification of Kelser's technique; the test was negative in a 1:5 serum dilution.

Examinations of the citrated blood consisted of wet-mount and Giemsa-stained preparations, as well as several cultures on Kelser's blood agar slants. Twenty-one examinations were made extending over a period of 257 days.

Cultural forms (leishmania-like forms, crithidia, and metacyclic stages) of the trypanosome were observed in varying numbers in the stored blood over a period of 35 days. On the 43rd day of storage no motile forms were observed in a wet-mount preparation; 0.5 ml of the blood was cultured on Kelser's blood agar slant (27° C). This culture was positive in seven days. Likewise, on the 55th and 144th days of storage no motile forms were observed on direct examination, but positive cultures were obtained. On the 172nd and 257th days of storage motile forms were fairly numerous in the wet-mount preparation. A Giemsa-stained smear of the blood at this time showed that the leishmania-like forms and crithidia were more abundant than the metacyclic stages.

Observations on *T. cruzi* in citrated blood collected early in the infection and stored at room temperature for 257 days, revealed that the trypanosomes not only remained viable but actually multiplied in the blood. It was concluded that citrated blood for the diagnosis of Chagas' disease is satisfactory (by direct examination and culture), even when stored at room temperature, for 257 days, or possibly longer.—THELMA D. SULLIVAN, *Division of Parasitology, Texas State Dept. of Health Laboratories, Austin, Texas.*

## NOTES ON ANOPLURA INFESTING MARINE CARNIVORES

From a total of 200 autopsies on seals at the San Diego Zoological Hospital, five incidents of pediculosis have been noted. The hosts include the California sea lion, *Zalophus californianus* Lesson, the Stellar sea lion, *Eumetopias jubata* Schreber and the Harbor seal, *Phoca richardii* Gray.

The lice were not restricted to any particular area on the body of the host; some being found on the head, others on the back and ventral surface. In no case observed was the infestation very heavy. However, all specimens taken were dead at the time of their recovery since the host in each case had unfortunately been placed in refrigeration prior to autopsy.

The lice were identified by the U. S. Dept. of Agriculture as *Antarctophthirus microchir* (Trous.). They belong to the subfamily Antarcticophthiriinae, which contains but a single genus *Antarctophthirus* Enderlein (Syn. *Arctophthirus* Mjoberg, 1910).

These lice are large, leathery and spine-studded and are of interest particularly from the standpoint of securing their oxygen supply. Ewing (1929) states, "Perhaps the lice of the family Echinophthiriidae get their air at the same intervals with their hosts when the latter come to the surface of water to breathe."

In the cases cited above, nits were not observed but nymphs and larger males and females were collected.—WILLIS H. DOETSCHMAN, *Biological Research Institute, San Diego, Calif.*

THE OCCURRENCE OF *AMBLYOMMA AMERICANUM* IN MINNESOTA AND IN OHIO

To the valuable data of Cooley and Kohls (1944, J. Parasitol. 30: 77-111) on the distribution of species of *Amblyomma* in the United States there may be added two previously unpublished records of the occurrence of *A. americanum*, indicating the presence of the species in Minnesota and in Ohio.

On June 4, 1942, I received from a resident of Winona, in southeastern Minnesota a specimen described as "a bug I have found on my person, under my arm. I noticed something there and thought it was a scab, so it remained there for six days." The sender was unable to supply any further significant information. The specimen was a headless ♀, but unquestionably *A. americanum*. No other records for Minnesota are known but there has been no intensive collecting of ticks.

The occurrence of *A. americanum* in southern Ohio would not be surprising in the light of distributional data but apparently it has not been reported for that state. I am obligated to Dr. J. H. Hughes, of the Public Health Service for permission to include in this note the fact that on April 26, 1939, he picked a ♀ specimen from his back, at Jackson, in southern Ohio. He was at the time interested in the ticks of Ohio and recognized the specimen as *A. americanum*. The identification was subsequently verified by Dr. J. C. Bequaert.—WILLIAM A. RILEY, *Division of Entomology and Economic Zoology, University of Minnesota*.

#### A SIMPLE PERMANENT MOUNTING METHOD FOR *NECATOR AMERICANUS*

Zwemer (1933, Anat. Rec. 57: 41-44) introduced the method of mounting frozen sections in glychrogel. Wotton (1936, Proc. Staten Island Inst. Arts and Sciences 8: 8-12 and 1937, Stain Technology 12: 145-146) has employed the method for mounting trematodes and other small animals. The following glychrogel formula has been used in the preparation of whole mounts of *Necator americanus*:

10% Bacto Gelatin, granular (Difco) .....	150 cc.
Glycerin, reagent (Merck) .....	50 cc.
1% Chromium and Potassium Sulfate, C.P. granular (Merck) .....	100 cc.
Phenol, U.S.P., liquid .....	1 cc.

The gelatin is dissolved in boiling water and the glycerin added. After mixing, the chrome alum solution and phenol are added. The medium may gel at room temperature but liquefies in 15 minutes at 65°. Specimens may be transferred from glycerin or formalin solutions to the mounting medium. Slides prepared in this way are ready for examination after 18 hours. The mounting medium hardens into a permanent mount; the specimens remain clear, with mouth parts and genitalia well defined. The method is especially advantageous for use with small nematodes and their ova.—I. JACQUES YETWIN, *Middlesex University School of Medicine, Waltham, Mass.*

#### A HERMIT CRAB AS INTERMEDIATE HOST OF *POLYMORPHUS* (ACANTH.)

Various crustaceans, Amphipoda predominantly, have been reported as intermediate hosts for Acanthocephala, but only two species of Decapoda have thus far been involved—the shrimp *Palaemon squilla*, host of *Arhythmorhynchus roseus*, and the crayfish *Potamobius astacus*, host of *Polymorphus minutus*. In Meyer's table (1933, Bronns Klassen und Ordnungen des Tierreichs., Bd. 4, Abt. 2, p. 340) of known invertebrate hosts for Acanthocephala, the listing of *Calliopius rathkei* under the Decapoda is erroneous since the animal is actually one of the Amphipoda.

During August and September, 1943, at Woods Hole, Mass., I examined more than 8,000 hermit crabs of the species *Pagurus longicarpus* Say and found that in addition to being parasitized by two species of Epicaridea the crabs were also infected with a larval acanthocephalid. The later was identified by John T. Lucker, Bureau of Animal Industry, as a species of the genus *Polymorphus* Lühe, 1911. Dr. Harley J. Van Cleave, to whom the specimens were later submitted, confirmed the generic identification and stated that a specific determination could not be made with reasonable certainty unless mature worms could be secured.

*Polymorphus* occurs in encysted condition in the abdominal cavity of the hermit crab. Usually the cysts are firmly attached to the outer wall of the mid- or hind-gut, but occasionally they occur among the tubules of the hepato-pancreas. One cyst per host is the usual condition but two or three were sometimes found in the same crab. Approximately one per cent of the hermit crabs from Great Harbor were infected with this acanthocephalid, but no case of simultaneous parasitism involving both Epicaridea and Acanthocephala was noted.

In shape the cysts of *Polymorphus* are oval or fusiform. They average 2.59 mm. in length (maximum 3.12, minimum 2.16) and 1.27 mm. breadth (maximum 1.44 minimum 1.20). Because of their relatively large size and glistening whiteness their presence can be detected without dissection of the crab. The body wall of the host's abdomen is transparent enough to reveal the cysts when the living crabs are examined under a low-power binocular dissecting microscope.—EDWARD G. REINHARD, *Catholic University of America, Washington, D. C.*

*GIARDIA SANGUINIS* (GONDER, 1911) NOT FROM A "FALCON"

Hegner and Taliaferro (1925, Human Protozoölogy) and Wenyon (1926, Protozoölogy) state that Gonder (1911, Arch. f. Protist. 21) described a *Giardia* from the blood of a "falcon." This declaration has been perpetuated in a number of references, the most recent of which is by Herman (1943, J. Parasit. 29). There is here a slight misimplication, however, which may be worth correcting.

Gonder's *Giardia* came from "ein blauer Falke, *Elanus coeruleus*." Now "falke" may mean falcon, hawk or kite, but *E. coeruleus* is neither falcon nor hawk. It is a kite (Accipitridae), and taxonomically is not even in the same family with the falcons (Falconidae). As continued reference to this host as a falcon leads to confusion ornithologically, it seems more appropriate to call Gonder's bird what it was, a kite.—ROBERT M. STABLER, Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.

*XENOPSYLLA CHEOPIS* IN LINCOLN, NEBRASKA

Two fleas (♀ and ♂) taken from an immature house rat, *Rattus norvegicus* (Erxleben), at Lincoln, Nebraska, December 28, 1943, have been identified as *Xenopsylla cheopis* Roths. The rat was collected in the basement of a home in Lincoln. Several specimens confirmed as *X. cheopis* were collected from a rat in Lincoln about eight years ago by parasitology students at the University of Nebraska, but exact data on this collection are not available. These records indicate that probably the species has become established in this area. Further efforts will be made, as circumstances permit, to determine the extent to which it occurs here.

It is not surprising to find *X. cheopis* in this area since it was reported from Ames, Iowa, in 1934, and Manhattan, Kansas, in 1941.—DORIS B. GATES, Department of Entomology, Nebraska Agricultural Experiment Station, Lincoln, Neb.

AMERICAN SOCIETY OF PARASITOLOGISTS  
THIRTY-SECOND COUNCIL MEETING, BALTIMORE, MARYLAND

JANUARY 29, 1944

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. Henry E. Meleney, President of the Society, at 2:00 PM, January 29, 1944, in the Department of Parasitology, Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Maryland. Past-president W. W. Cort and the following members of the Council were present: D. L. Augustine, E. R. Becker, H. E. Meleney, G. F. Otto, E. W. Price, L. E. Rozeboom, H. W. Stunkard, and W. H. Wright.

I. REPORTS OF OFFICERS

1. *Secretary (J. T. Culbertson)*: As of December 31, 1943, there were 520 persons on the membership roll of the Society, of whom 454 lived within and 66 lived outside continental United States. Of those on the roll, 419 were members in good standing and 101 were delinquent in dues for either 1 or 2 years. Of those in good standing, 380 lived within and 39 lived outside continental United States. Twenty-eight persons were elected to membership during the year, of whom 25 lived within and 3 lived outside continental United States. One member died during the year: Dr. William A. Hoffman, Assistant Professor of Parasitology, Columbia University, School of Tropical Medicine, San Juan, Puerto Rico.

Upon motion, the Secretary's report was accepted and placed on file.

2. *Treasurer (L. E. Rozeboom)*: For the fiscal year 1943 (Dec. 7, 1942 to Dec. 6, 1943):

*Receipts*

Balance on hand, Dec. 7, 1942 (J. Parasitol. 29: 236) .....		\$2525.15
Collected during current year		
Member dues applying 1943 .....	\$ 756.51	
Member dues applying 1944 .....	440.00	
Advance dues .....	8.00	1204.51
Subscriptions applying 1943 .....	1719.55	
Subscriptions applying 1944 .....	377.00	
Advance subscriptions .....	11.25	2107.80
Back vols. and nos. sold .....	189.25	
Arrearages .....	52.00	
25-vol. index sales .....	59.40	
Contributions from members .....	89.00	
Advertisements .....	190.00	
Author charges .....	91.00	
Miscellaneous .....	27.87	698.52
Grant from Amer. Found. Trop. Med. ....		1000.00
		<hr/> \$7535.98

*Expenditures*

Printing journal		
1942 account .....	\$ 829.04	
1943 account .....	2698.15	
		<hr/> \$3527.19
Expenses in office of Chm. Ed. Comm. ....	147.44	
Expenses in office of Secretary .....	102.77	
Expenses in office of Treasurer .....	324.52	
Payment of loan from Princeton Fund .....	402.00	
Miscellaneous .....	74.90	
		<hr/> 4578.82
Balance on hand, Dec. 6, 1943 .....		\$2957.16



Upon motion, the Treasurer's report was accepted, subject to audit, and placed on file.

3. *President (H. E. Meleney)*: Correspondence with Dr. H. B. Ward with respect to back numbers of the JOURNAL OF PARASITOLOGY had revealed that the number of copies of early volumes now in Dr. Ward's possession was not large enough to justify his transferring them to the Society.

During the last several weeks, the members of the Society were canvassed to determine their participation in war activities. The returns are now being studied and an analysis of the findings will be published in the JOURNAL OF PARASITOLOGY in the near future.

## II. REPORTS OF COMMITTEES

### 1. *Custodian of the Princeton Secretarial Fund (N. R. Stoll)*:

	Dec. 3, 1943	One year ago
A. Total liquid assets .....	\$998.55	\$ 475.79
B. Book value subsidiary assets .....	None	816.67
Total .....	998.55	1292.46

Upon motion, the report was accepted, subject to audit, and placed on file. A vote of thanks was extended to the Custodian for his careful supervision of the Fund.

2. *Chairman of the Editorial Committee (N. R. Stoll)*: During 1943, the six regular numbers of volume 29 of the JOURNAL OF PARASITOLOGY containing 444 pages (equivalent to 555 pages of the earlier format) were published. Sixty-five regular articles and 28 research notes were included in the volume. The interval between "received for publication" and date published was, for the regular articles, 9.2 months (compared with 11.0 months in 1942) and, for research notes, 8.6 months (compared with 10.3 months in 1942). The year was marked by a continuation of the trend, noted earlier, of a falling off in submitted manuscripts. The entrance of many members of the Society into the armed forces was considered the chief reason for fewer manuscripts being received.

During 1944, by order of the War Production Board, only 90 per cent, by weight, of the paper used in 1942 can be obtained for printing the JOURNAL. Fortunately, because of the new format, this reduction in available paper will not reduce the amount of material published.

During the year, with approval of the Editorial Committee and Board, and of Council, application was made to the American Foundation for Tropical Medicine for a subvention of \$1,000 to aid in publishing the JOURNAL OF PARASITOLOGY, on condition "that the Foundation would grant it without restriction, use of the money to be at the discretion of the Committee, present standards of the JOURNAL OF PARASITOLOGY to be upheld." The Foundation promptly granted the sum, thus permitting the Editorial Committee to relax its charge on authors when this was less than \$5.00, as well as its 10-page limit on the size of articles. Actually, the Foundation grant was unused at year-end and was turned over intact to the new Editorial Committee.

During 1943 there was a total of 822 paid members and subscribers, 3 less than in 1942. Especially heartening as a sign of loyalty to the JOURNAL was the voluntary contribution of \$313.00 by 145 dues-paying members during the year.

During the year, there were 31 exchanges on the JOURNAL list. This number could easily be doubled, at least when the war ends, without creating a serious financial burden.

Thirty copies of the 25-year Index were sold in 1943, bringing the total number sold to 400 of the 1200 printed. Consideration is invited of the judicious distribution of the Index to libraries in South and Central America and after the war ends to devastated libraries in war areas, perhaps through the American Library Association Committee.

Sets of volume 29 of the JOURNAL were purchased by the American Library Association Committee for devastated libraries in war areas.

Upon motion the report was accepted and placed on file. A vote of thanks was extended to Dr. Stoll and the Editorial Committee for its services, as well as to the Rockefeller Institute at Princeton for supplying clerical service afforded to Dr. Stoll while Chairman of the Editorial Committee.

3. *Committee to Audit Reports of Treasurer and Custodian of Princeton Secretarial Fund (G. F. Otto, W. W. Cort)*: The Auditing Committee approved the reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund.

Upon motion, the Committee's report was accepted and placed on file.

## III. NEW BUSINESS

1. *New Members*: Council voted membership in the Society upon two applicants: Dallas K. Meyer, Aquatic Biologist, United States Fish and Wildlife Service, Ft. Worth, Texas, and William Cleland Forbes, King's College, New Castle, Delaware.

2. *Management of the Journal*: Council voted to give the Editorial Committee power to decide its own policy for increasing the number of submitted manuscripts or the size limit of acceptable manuscripts.

3. *Selection of Place for the 1944 Meeting of the Society*: No decision was reached as to time or place of meeting in 1944.

4. *Election of Officers, Appointments, etc.*

- a. Council voted to confirm the mail vote by members of the Society which was taken late in 1943. Accordingly, the following were elected to the Society's offices: Henry E. Ewing, *President* for one year; Benjamin Schwartz, *Vice-President* for one year; L. E. Rozeboom, *Treasurer* for one year; J. T. Culbertson, *Secretary* for two years; H. W. Manter and E. W. Price, *Councillors-at-Large* for three years; and C. G. Huff and H. W. Brown, *Councillors-at-Large* for four years.
- b. The following persons were elected by Council to the Editorial Board of the JOURNAL OF PARASITOLOGY: L. T. Coggeshall, J. T. Lucker, and N. R. Stoll.
- c. *Committee on Terminology of Strains of Avian Malaria*: Council voted to continue the Committee (C. G. Huff (Chairman), G. H. Boyd, and R. D. Manwell) for one year and recommended that a supplement to the last report of its activities be published.
- d. *Committee on Nomenclature*: The present Committee, consisting of E. W. Price (Chairman), B. G. Chitwood, and A. McIntosh, was reappointed, with power to represent the Society on the General Council on Zoological Nomenclature.
- e. *Committee on Common Names of Parasites*: A committee to decide on acceptable common names of parasites of man and other animals was erected. P. D. Harwood was named Chairman and two other members were to be selected later.
- f. *Representatives on the Council of the American Association for the Advancement of Science*: A. C. Chandler and H. J. Van Cleave were named as representatives for one year.
- g. *Representatives on the Council of the Union of American Biological Societies*: A. O. Foster and A. C. Walton were named as representatives for one year.
- h. *Inactive or Suspended Membership*: Council voted that members of the Society who, because of the war, are outside continental United States or temporarily out of touch with their usual parasitological activity are to be given inactive or suspended status if they become delinquent in payment of dues. They are not to be dropped from the Society membership roll for the duration of the war. They can resume active membership at any time on payment of current dues, and on payment of delinquent dues may receive back numbers of the JOURNAL.

Council voted its thanks to the Society Officers and Representatives and adjourned.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary*.

## SECOND REPORT OF THE COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

The proposal made by this committee in the supplement to the report of the 17th annual meeting of the American Society of Parasitologists (J. Parasitol. 28: 250, 1942) that a uniform terminology of strains of avian malaria be adopted has met with uniformly favorable reactions on the part of investigators. The designations of strains and substrains made then have been widely used. The committee holds the opinion that this practice is aiding in preventing confusion in the terminology applied to strains of avian malaria and recommends its continuance. Designations made subsequent to the previous report are listed in tables 1 and 2. For an explanation of the principles guiding the committee in applying the designations reference is made to the previous report, Jour. Parasitol. 28: 250-254, June, 1942.

Again it is urged that investigators use the official designations of strains when publishing the results of their experiments or when sending the strain to others. The committee will be pleased to make designations of new strains or substrains prior to the publication of experimental work upon such strains. We wish to express our appreciation for the cooperation shown already on the part of all investigators involved. We are also pleased to extend our service to scientists in other countries.

### COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

CLAY G. HUFF, *Chairman*  
GEORGE H. BOYD  
REGINALD D. MANWELL

TABLE 1.—Designations of original strains

Designation	Host source	Host locality	Isolated by	Date of isolation	Other names used	Reference
<i>P. reticulum</i> 1 C	<i>Escafactoria lineata</i> (Island painted quail)	Philippine Is.	P. F. Russell	1932	<i>P. capistrani</i>	Phil. J. Sci. 48: 269 (1932). (Designation requested by Dr. F. Woll- son, Amer. J. Trop. Med. 16: 685 (1936))
<i>P. cathemerium</i> 3 C	<i>Passer domesticus</i>	Oshkosh, Wis.	P. E. Thompson	1944		None
<i>P. elongatum</i> 5 C	<i>Sialia s. sialis</i> (Bluebird)	Atlanta, Ga.	W. B. Redmond	1942		None
<i>P. elongatum</i> 5 D	<i>Passer domesticus</i>	Indianapolis, Ind.	E. Price	1943		None
5 M	<i>Sturnella magna</i> (Meadowlark)	Kansas, Ill.	C. G. Huff	1934		J. Inf. Dis. 57: 315 (1935)
<i>P. circumflexum</i> 6 F	<i>Passer domesticus</i>	Oshkosh, Wis.	P. E. Thompson	1944	ML	None
<i>P. juatanucleare</i> 14 A	<i>Gallus domesticus</i>	Western part of Minas Geraes, Brazil	Versiani and Gomes	1941		Rev. Brasil. Biol. 1: 231 (1941)
14 B	<i>Gallus domesticus</i>	Huixtla, Chiapas, Mexico	E. Beltran	1942		Rev. Inst. Salud, y. Enfr. trop. 2: 353 (1941)



TABLE 2.—Designations of sub-strains

Designation	Source	Derivation and investigator	Characteristic	Reference
1P1 ( <i>P. relictum</i> )	1P, Coatney's pigeon strain	W. B. Redmond	Adapted to blood infection in canary. Infects <i>Culex</i> mosquitoes	Bull. Ga. Acad. Sci. 1: 9 (1943). J. Inf. Dis. 74: 184-188
1P1-1 ( <i>P. relictum</i> )	1P1	Through mosquito passage by W. B. Redmond	No longer infects pigeon either by inoculation of blood or sporozoites or by bite of infected mosquito	Bull. Ga. Acad. Sci. 1: 9 (1943). J. Inf. Dis. 74: 184-188
3H1-3 ( <i>P. cathemerium</i> )	3H1-2 or "p" strain	Treated in vitro with high sub-lethal temperatures by F. E. Caldwell	Shows marked disturbance in synchronism of schizogony	J. Inf. Dis. 74: 189-205
3H1-4 ( <i>P. cathemerium</i> )	3H1-2 or "p" strain	Treated in vitro with high sub-lethal temperatures by F. E. Caldwell	Shows marked disturbance in synchronism of schizogony and loss of gametocytes. No longer capable of transmission by mosquitoes	J. Inf. Dis. 74: 189-205
3H1-5 ( <i>P. cathemerium</i> )	3H1-2 or "p" strain	Treated in vitro with high sub-lethal temperatures by F. E. Caldwell	Not noticeably changed from parent strain	J. Inf. Dis. 74: 189-205
12A1 ( <i>P. lophurae</i> )	12A	Adapted to canary by F. Coulston	Capable of producing heavy infections in canaries	None

# The Journal of Parasitology

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## PROGRAM AND ABSTRACTS OF THE NINETEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

CLEVELAND, OHIO

SEPTEMBER 11 and 12, 1944

### PROGRAM<sup>1</sup>

MONDAY MORNING SESSION, SEPTEMBER 11, 9:00 A. M.; ROOM 23, CHEMISTRY  
BUILDING, WESTERN RESERVE UNIVERSITY.

#### Read

1. Effects of Various Sulpha Drugs on the Protozoan Parasite *Eimeria tenella*. (15 min) (Lantern) CHARLES A. RIPSOM, University of Wisconsin.
2. Sulfamethazine in the Treatment of *Eimeria tenella* Infections in Poultry. (10 min) (Lantern) PHILIP A. HAWKINS, Michigan State College.
3. The Effect of Some Aryl Arsonic Acids on Experimental Coccidiosis Infection in Chickens. (15 min) (Lantern) NEAL F. MOREHOUSE AND ORLEY J. MAYFIELD, Dr. Salsbury's Laboratories, Charles City, Iowa.
4. Experimental Studies on the Life Cycle of *Eimeria bovis* in Calves. (15 min) (Lantern) DATUS M. HAMMOND, LEONARD REID DAVIS, GEORGE W. BOWMAN AND BENNETT T. SIMMS, United States Bureau of Animal Industry.
5. Physiological Responses of *Neoechinorhynchus emydis* (Acanthocephala) to Various Solutions. (15 min) HARLEY J. VAN CLEAVE AND ELIZABETH L. ROSS, University of Illinois.
6. Further Notes on *Chabertia ovina*. (15 min) (Lantern) W. L. THRELKELD, Virginia Agricultural Experiment Station.
7. Studies on a New Xiphidiocercaria of the Virgula Type with Notes on the Life History. (10 min) (Lantern) PHILIP G. SEITNER, Purdue University.
8. Motion Picture of *Cercaria clausii* Monticelli, a Marine Rattenkönig Larval Trematode from the West Coast of Florida. (5 min) (Motion picture) R. M. CABLE, Purdue University, AND RICHARD A. MCLEAN, Academy of Natural Sciences of Philadelphia.
9. *Histoplasma capsulatum*, Its Biochemical and Immunological Properties. (15 min) GEORGE J. SCHEFF, Ohio State University.
10. The Incidence of Intestinal Protozoa in Mental Patients and in Cases Showing Symptoms of Amoebic Dysentery. (15 min) VIOLA M. YOUNG, Chicago Medical School and the Chicago Free Dispensary.

<sup>1</sup> An alphabetical author index will be found at the end of this program. Extra copies of this Supplement, and portraits of parasitologists, will be on sale at the meeting.

11. On the Staining of Protozoa in Human Blood and Feces. (15 min)  
OSCAR FELSENFELD, Chicago Medical School and the Chicago Free Dispensary.

12. The Occurrence of the Pentastomid, *Porocephalus crotali* Humbolt, in Rattlesnakes of Southwestern Oklahoma. J. TEAGUE SELF, University of Oklahoma.

#### By Title

13. Paramphistomes of North American Domestic Ruminants. EMMETT W. PRICE AND ALLEN MCINTOSH, United States Bureau of Animal Industry.

14. Trypanosomes in North American Amphibians. ROSS F. NIGRELLI, New York Zoological Society.

15. A Blood Parasite of the Turkey. E. MCNEIL AND W. R. HINSHAW, University of California.

16. The Continuous Culture of Pure Clones of *Dero limosa* Infected with *Ade-  
lina deronis*. THEODORE S. HAUSCHKA, Lankenau Hospital Research Institute.

17. A Case of *Trichostrongylus* sp. with Notes on the Identification of Ova.  
H. TSUCHIYA AND HELEN RELLER, Washington University (St. Louis, Mo.).

18. The Production of Anemia in Lambs by Hookworms, *Bunostomum trigono-  
cephalum*. JOHN T. LUCKER AND ELEANOR M. NEUMAYER, United States Bureau  
of Animal Industry.

MONDAY AFTERNOON SESSION, SEPTEMBER 11, 2:00 P. M.; ROOM 23, CHEMISTRY  
BUILDING, WESTERN RESERVE UNIVERSITY.

#### Read

19. Comparative Growth Rates of Amoebae and Bacteria in Cultures of *Enda-  
moeba histolytica* and Organism *t*. (15 min) (Lantern) CHARLES W. REES AND  
LUCY V. REARDON, National Institute of Health.

20. Inoculations of *Trichomonas foetus* (Protozoa) in Heifers. (15 min)  
(Lantern) BANNER BILL MORGAN, University of Wisconsin.

21. The Action of Negro and White Sera on Infective Hookworm (*Necator  
americanus*) Larvae. (15 min) Lantern) GILBERT F. OTTO, EVELYN ALLEN  
AND STERLING BRACKETT, Johns Hopkins University.

22. Tests to Determine the Ability of Species of Domestic Mosquitoes to Trans-  
mit *Wuchereria bancrofti*. (10 min) WALTER L. NEWTON AND WILLARD H.  
WRIGHT, National Institute of Health.

23. Agglutination of Microfilariae from *Litomosoides carinii* of the Cotton Rat  
by Normal Serums of Man and Other Animals. (15 min) (Lantern) JAMES  
T. CULBERTSON AND HARRY M. ROSE, Columbia University.

24. Cross Reactions between Polysaccharides from Various Animal Parasites  
(15 min) JOSÉ OLIVER-GONZÁLEZ, School of Tropical Medicine, Puerto Rico.

25. The Effects of Host Starvation on Worm Elimination and Glycogen Deple-  
tion with the Nematode *Ascaridia galli*. (10 min) (Lantern) W. MALCOLM  
REID, Monmouth College.

26. Substitution of Soybean Oil Meal for Animal Protein in Developing Re-  
sistance of Animals to Parasitism. (10 min) (Lantern) J. E. ACKERT, DORO-  
THY S. BRANSON, D. J. AMEEL, AND B. B. RIEDEL, Kansas State College.

27. Studies on Effects of Helminths on Growing Chickens. (10 min) (Lantern) J. E. ACKERT AND C. L. WISSEMAN, JR., Kansas State College.
28. The Effects of Nematode Infections During the Prepatent Period on the Calf. (15 min) ROY L. MAYHEW, Louisiana State University.
29. Alcoholism in Mice and its Effect on Natural and Acquired Resistance to *Hymenolepis nana* var. *fraterna*. (15 min) JOHN E. LARSH, JR., University of North Carolina.
30. Critical Tests with a Hexachloroethane-bentonite-water Suspension as a Fasciolicide in Cattle. (15 min) O. WILFORD OLSEN, U. S. Bureau of Animal Industry.
31. Common Names of Helminths. (15 min) DONALD B. McMULLEN, University of Oklahoma; H. L. VAN VOLKENBERG, Texas Agricultural and Mechanical College; AND PAUL D. HARWOOD, Drs. Hess and Clark Laboratories, Inc., Ashland, Ohio.

*By Title*

32. A Reaction of Rat Serum to Embryonated Eggs of *Trichosomoides crassicauda*. VIVIAN S. SMITH, University of Illinois.
33. Studies on the Relationship of *Trichosomoides crassicauda* Infection in Rats to Mucoïd Calculi of the Urinary Bladder. VIVIAN S. SMITH, University of Illinois.
34. The Possibility of Eliminating the Schistosome Snail, *Australorbis glabratus*, from Canals by Controls of Water Flow. GEORGE W. LUTTERMOSER, Oficina Co-operativa Inter-Americana de Salud Publica (Venezuela).
35. The Parasites of the Plethodontidae (Amphibia-Caudata) III. ARTHUR C. WALTON, Knox College.
36. The Parasites of the Plethodontidae (Amphibia-Caudata) IV. ARTHUR C. WALTON, Knox College.
37. The Parasites of the Proteidae and the Sirenidae (Amphibia-Caudata). ARTHUR C. WALTON, Knox College.
38. Effect of Skim Milk on Infections of Ascarids, Whipworms, and Nodular Worms in Swine. L. A. SPINDLER AND HARRY E. ZIMMERMAN, JR., United States Bureau of Animal Industry.
39. An *in vitro* Method for the Bio-assay of Chemotherapeutic Agents in Filariasis. HARRY M. ROSE, JAMES T. CULBERTSON AND ELEANORA MOLLOY, Columbia University.

TUESDAY, SEPTEMBER 12.

1:00 P.M. PARASITOLOGISTS' LUNCHEON.

2:30 P.M. PRESIDENTIAL ADDRESS.

40. The Trombiculid Mites (Chigger Mites) and Their Relation to Disease. HENRY E. EWING, United States Bureau of Entomology and Plant Quarantine.

3:00 P.M. ANNUAL BUSINESS MEETING.



## AUTHOR INDEX

Showing program number, which is also the abstract number of each paper.

<i>Author</i>	<i>Program and Abstract Number</i>	<i>Author</i>	<i>Program and Abstract Number</i>
Ackert, J. E. ....	26, 27	Newton, W. L. ....	22
Allen, E. ....	21	Nigrelli, R. F. ....	14
Ameel, D. J. ....	26	Oliver-González, J. ....	24
Bowman, G. W. ....	4	Otto, G. F. ....	21
Brackett, S. ....	21	Olsen, O. W. ....	30
Branson, D. S. ....	26	Price, E. W. ....	13
Cable, R. M. ....	8	Reardon, L. V. ....	19
Culbertson, J. T. ....	23, 39	Rees, C. W. ....	19
Davis, L. R. ....	4	Reid, W. M. ....	25
Ewing, H. E. ....	40	Reller, H. ....	17
Felsenfeld, O. ....	11	Riedel, B. B. ....	26
Hammond, D. M. ....	4	Ripsom, C. A. ....	1
Harwood, P. D. ....	31	Rose, H. M. ....	23, 39
Hauschka, T. S. ....	16	Ross, E. L. ....	5
Hawkins, P. A. ....	2	Scheff, G. J. ....	9
Hinshaw, W. R. ....	15	Seitner, P. G. ....	7
Larsh, J. E., Jr. ....	29	Self, J. T. ....	12
Lucker, J. T. ....	18	Simms, B. T. ....	4
Luttermoser, G. W. ....	34	Smith, V. S. ....	32, 33
Mayfield, O. J. ....	3	Spindler, L. A. ....	38
Mayhew, R. L. ....	28	Threlkeld, W. L. ....	6
McIntosh, A. ....	13	Tsuchiya, H. ....	17
McLean, R. A. ....	8	Van Cleave, H. J. ....	5
McMullen, D. B. ....	31	Van Volkenberg, H. L. ....	31
McNeil, E. ....	15	Walton, A. C. ....	35, 36, 37
Molloy, E. ....	39	Wisseman, C. L., Jr. ....	27
Morehouse, N. F. ....	3	Wright, W. H. ....	22
Morgan, B. B. ....	20	Young, V. M. ....	10
Neumayer, E. M. ....	18	Zimmerman, H. E., Jr. ....	38

SYMPOSIUM ON PARASITOLOGY IN  
RELATION TO THE WAR

Organized in Cooperation with Section N (Medical Sciences) of the A.A.A.S., the  
American Society of Zoologists, and the American Society of Tropical Medicine.

CLEVELAND, OHIO

TUESDAY MORNING, 9:30 A.M., SEPTEMBER 12, 1944, THE AUDITORIUM, ACADEMY  
OF MEDICINE.

PROGRAM

1. The Wartime Importance of Tropical Diseases. JAMES S. SIMMONS, Brig.  
General, Medical Corps, U. S. Army.
2. Malaria and The War. O. R. MCCOY, Major, Medical Corps, U. S. Army.
3. The Development of Malarial Sporozoites in the Vertebrate Host. CLAY G.  
HUFF AND FREDERICK COULSTON, University of Chicago.
4. The Present Status of the Filariasis Problem. H. W. BROWN, Columbia  
University.
5. Distribution and Control of the Fevers of the Typhus group. R. E. DYER,  
National Institute of Health.
6. Development of Louse Powders for the Armed Forces. W. E. DOVE, U. S.  
Bureau of Entomology and Plant Quarantine.
7. Parasites in Relation to Production of Meat and Other Animal Products in  
Wartime. BENJAMIN SCHWARTZ, U. S. Bureau of Animal Industry.

## ABSTRACTS

## AMERICAN SOCIETY OF PARASITOLOGISTS' PROGRAM

1. *Effects of Various Sulpha Drugs on the Protozoan Parasite, Eimeria tenella.* CHARLES A. RIPSOM, University of Wisconsin.

Various sulpha drugs have been used to determine their effect on the protozoan parasite, *Eimeria tenella*, causing caecal coccidiosis in chickens. Tests carried out so far demonstrate that sulphadiazine will not only prevent development of the asexual stages of the parasite but will also prevent the development of the sexual stage. This compound has been found effective when given in single doses or when administered in the feed.

2. *Sulfamethazine in the Treatment of Eimeria tenella Infections in Poultry.* PHILIP A. HAWKINS, Michigan State College.

Four-week-old chicks infected with *Eimeria tenella* were treated with sulfamethazine at the first appearance of blood in the droppings. The drug was administered to one group of twenty birds as a saturated solution, to a similar group as 1 per cent of the feed. The mortality of the untreated controls was 78 per cent, of those receiving the drug in the water 70 per cent, and of those receiving it in the feed 10 per cent. The average sulfamethazine level in the blood of birds dying while receiving the drug in water and feed was 1.2 mg per cent and 0.5 mg per cent, respectively. The blood levels of the same groups surviving the infection were 3.2 mg per cent and 16.3 mg per cent for a four-day period after treatment started. Similar groups of uninfected birds given sulfamethazine in the water and feed had blood levels of 4.4 mg per cent and 20.2 mg per cent, respectively. Infected birds will continue to eat longer than they will drink, while some groups of infected birds will drink more than others. This probably explains the discrepancy between these results and those previously reported by the writer (1943, Poultry Sci. 22: 459).

3. *The Effect of Some Aryl Arsonic Acids on Experimental Coccidiosis Infection in Chickens.* NEAL F. MOREHOUSE AND ORLEY J. MAYFIELD, Dr. Salsbury's Laboratories, Charles City, Iowa.

The aryl arsonic acids 4-hydroxy phenylarsonic acid, 3-nitro 4-hydroxy phenylarsonic acid and their sodium salts, when given at relatively non-toxic doses prior to infection, have repeatedly reduced or completely eliminated hemorrhage and mortality in test groups of chickens infected with the coccidium *Eimeria tenella*. A preparation containing sodium 4-hydroxy phenylarsonate, sodium and ammonium phenolsulphonates, boric acid and lactose has shown high efficacy when the concentration of the arsenic compound in water was 0.0269 per cent. In three experiments using 252 treated chicks and 252 controls, the mortality from cecal coccidiosis was 2 and 83 respectively. Similar results have been obtained with phenolsulphonate-lactose-boric acid preparations containing 3-nitro 4-hydroxy phenylarsonic acid where the concentration of the arsenic compound in the water was much lower than was necessary when 4-hydroxy phenylarsonic acid was used. When the concentration of 3-nitro 4-hydroxy phenylarsonic acid in such preparations was approximately 0.00256 per cent in the water, marked growth-stimulating properties were noted. In numerous experiments statistical analyses have shown highly significant differences between the mean gains of treated and control chicks.

4. *Experimental Studies on the Life Cycle of Eimeria bovis in Calves.* DATUS M. HAMMOND, LEONARD REID DAVIS, GEORGE W. BOWMAN AND BENNETT T. SIMMS, U. S. Bureau of Animal Industry.

Small numbers of sporozoites were found in the lower small intestine of a 3-day-old calf killed 18 hours after inoculation with oöcysts of *Eimeria bovis* (syn. *E. smithi*). Some excystation occurred in oöcysts incubated for 24 hours in filtered intestinal contents, while oöcysts excysted partially in a cellophane bag placed for 18 to 46 hours in a Thiry fistula in a calf. The first stage found was identified as a schizont. Schizonts occurred chiefly in the lower small intestine. Each schizont was typically located at or near the center of a villus, in a host cell encapsulated by fibroblasts. The schizonts were found as early as five days after inoculation, but numerous schizonts were not found until 11 days after inoculation. The smallest schizont found measured 9.1 microns in greatest diameter; at maturity, beginning about 14 days after inoculation, the schizonts reached a maximum size of 450 microns, and contained thousands of merozoites. The number of generations has not been determined. That the merozoites may give rise to the sexual stages was shown in two trials, which resulted in the recovery of *E. bovis* oöcysts from the Thiry fistula, 5, and 4 to 16 days, respectively, after injection of schizonts and merozoites obtained from the intestine of calves 23 and 17 days, respectively, following inoculation of oöcysts. The sexual stages occur in the intestinal glands, usually only in the cecum and colon. The development of the macrogametocyte, microgametocyte, and oöcysts was observed.

5. *Physiological Responses of Neoechinorhynchus emydis (Acanthocephala) to Various Solutions.* HARLEY J. VAN CLEAVE AND ELIZABETH L. ROSS, University of Illinois.

Except for gravid females, bodies of living *Neoechinorhynchus emydis* in intestine of turtle hosts are normally flat and wrinkled. Subjection to the usual saline solutions for examination and routine preservation render them turgidly cylindrical. Since all metabolic materials must pass through the body wall, maintenance of normal permeability is extremely important.

Removed to water, swelling was conspicuous in one hour and death followed in a few hours. In 0.5 per cent sodium chloride with 0.02 per cent calcium chloride (Gettier, 1942) turgidity was delayed until the third or fourth day; worms lived 10 to 12 days *in vitro*. Only sample experiments are here cited; condition of worms and host are uncontrolled factors.

In 0.7 and 0.75 per cent sodium chloride, worms remained flat 4 days; survived about 10 and 12 days respectively. In 0.8 per cent sodium chloride, worms remained flat the full 10 days of survival. In 0.85 per cent, they became excessively wrinkled; lived 15 days; turgidity was postponed until thirteenth day.

After 1 hour in water, turgid worms transferred to 0.85 per cent sodium chloride resumed flattened condition in 5 hours; lived 5 to 8 days.

Solutions were administered by mouth to living turtles. One hour after injecting water, autopsy revealed highly turgid worms attached in intestine. 0.8 per cent sodium chloride, 4½ hours after introduction, yielded worms normally flattened, wrinkled. Removed to same solution *in vitro*, worms lived 10 days.

6. *Further Notes on Chabertia ovina.* W. L. THRELKELD, Virginia Agricultural Experiment Station.

A study is being made on *Chabertia ovina*, the large-mouthed bowel worm of sheep, in an effort to clarify its status in relation to its host animal. Two phases are at present considered. First, a preliminary study of a possible hemolytic factor, together with a detailed examination of the cephalic, cervical, and other anatomical structures in the parasite indicate further tests should be pursued. A table showing positive results from the action of the possible hemolytic factor against defibrinated sheep blood is presented, as well as photomicrographs illustrating an analysis of the structures which may be involved. The second phase involves observations made on lambs experimentally infested with pure cultures of the infective larvae of *Chabertia ovina*. Typical symptoms of parasitization have been established in two lambs. The records of these animals show loss of weight, profuse bloody diarrhea, and a significant drop in hemoglobin and erythrocytes.

7. *Studies on a New Xiphidiocercaria of the Virgula Type with Notes on the Life History.* PHILIP G. SEITNER, Purdue University.

As many as 30 per cent of the specimens of *Goniobasis depygis* collected from McCormick's Creek, Indiana, have been found to harbor a new stylet cercaria of the *Virgula* type. The larvae penetrate and encyst in mayfly naiads, becoming metacercariae identical with those occurring in naturally infected naiads from the same locality. The adult stage possibly may be *Loxogenes bicolor* which has been recovered from frogs collected from the stream; metacercariae show unmistakable adult lecitodendriid characters which correspond closely to those of *L. bicolor*. Feeding experiments are in progress. Measurements in mm of 10 cercariae killed in hot 10 per cent formalin: body length 0.137–0.167 (average 0.15), width 0.045–0.06 (0.053); tail length 0.068–0.091 (0.08), width near base 0.016–0.018 (0.017); length of oral sucker 0.04–0.05 (0.045), width 0.038–0.04 (0.039); ventral sucker 0.018–0.019 in diameter; stylet length 0.018–0.02 (0.019); diameter of pharynx 0.01. Entire body spinose, cuticle thick and with several hair-like processes near mouth; ventral sucker at beginning of posterior body half; tail simple, aspinose. Three pairs of cephalic glands behind anterior margin of ventral sucker; posterior pair granular; ducts in a single bundle on each side. *Virgula* organ large, trilobed in appearance when flattened; intestine undeveloped. Excretory vesicle U-shaped, surrounded by granular cells; main excretory tubules extend anterolaterad from arms of vesicle, dividing at level of ventral sucker into anterior and posterior tubules. Develop in small, oval sporocysts in the digestive gland of the snail.

8. *Motion Picture of Cercaria clausii Monticelli, a Marine Rattenkönig Larval Trematode from the West Coast of Florida.* R. M. CABLE, Purdue University, AND RICHARD A. McLEAN, Academy of Natural Sciences of Philadelphia.

A short motion picture of *C. clausii* illustrates the behavior of this rare type of larval trematode. In some sequences, the clusters of larvae move as units, all members contracting simultaneously, while in others, the individual cercariae move independently as if attempting to detach themselves from the rosette.



9. *Histoplasma capsulatum*, Its Biochemical and Immunological Properties. GEORGE J. SCHEFF, Ohio State University.

The biochemical and immunological properties of the mycelial form of *H. capsulatum* have been studied. The biochemical behavior is characterized by slow utilization of sugars and by an early formation of ammonia from any available nitrogen source. This is enhanced rather than inhibited by the presence of glucose. Organic nitrogen compounds (asparagine) due to their catalytically active carbon structure, are definitely superior as nitrogen sources to inorganic nitrogen sources (ammonia sulphate). The decomposition of the nutrients is brought about by the interaction of various enzymes and an energy-yielding cycle of carbon metabolism. The latter includes succinate and fumarate which, when added definitely accelerate the growth and increase the yield of the mold. The fungus is able to elaborate some of its growth factors but has to be supplied with thiamine from outside.

The immunological behavior is similar to that of other fungi. Due to the slow development of the infection, no humoral antibodies are produced, but a high degree of specific skin sensitivity is established. In immunized animals the reverse conditions seem to prevail. From the mold, potent polysaccharide and protein fractions can be obtained. These give skin tests with infected animals and precipitation tests with the sera of immunized animals.

The results were compared with those obtained with other pathogenic fungi and a tentative explanation of these findings is proposed.

10. *The Incidence of Intestinal Protozoa in Mental Patients and in Cases Showing Symptoms of Amoebic Dysentery*. VIOLA M. YOUNG, Chicago Medical School and the Chicago Free Dispensary.

The stool specimens from 1649 mentally ill patients in Illinois State Hospitals, 500 of whom were newly admitted, 800 chronically institutionalized, and 349 with diarrhea were examined and the results compared with findings in 361 mentally sane patients showing symptoms of amoebic dysentery.

From the 349 cases of diarrhea among hospital patients, 30 were bacillary in origin, 15 amoebic, 203 food poisoning, and 101 had diarrhea of unknown origin. Of the 361 outside patients, 157 had amoebic dysentery, and 204 were non-microbial and non-protozoan in origin. Fresh stools and non-cathartic and post-cathartic in nature were examined unstained, iodine stained and finally after permanent staining.

Mental patients showed an increase in number of all protozoa during hospitalization due to the poor hygienic habits of these cases. The numbers of specimens harboring *E. nana*, *I. butchlii*, and flagellates increased sharply during diarrhea. The incidence of *Chilomastix mesnili*, *Giardia lamblia*, and *Trichomonas hominis* was so high in the group of "diarrheas of unknown origin" in mentally ill patients, that their connection with acute intestinal disturbance must be considered. *E. nana* and *E. coli* were more numerous in the mentally sane patients suffering from diarrhea than among the institutionalized. The occurrence of *Enteromonas hominis* and *Embadomonas intestinalis* during the winter 1943-44 was high both in cases of amoebic dysentery and non-specific diarrhea. No explanation could be found for this phenomenon.

11. *On the Staining of Protozoa in Human Blood and Feces*. OSCAR FELSENFELD, Chicago Medical School and the Chicago Free Dispensary.

The desire to save expensive chemicals and valuable time led to a search for simple, quick and cheap staining methods.

The rapid method of Johnson gives results that are inferior to those of the classic Heidenhain or Dobell stain for intestinal amoebas. When, however, Carnoy's fixative precedes Johnson's method instead of the alcoholic sublimate, more brilliant pictures are obtained. Mann's stain is not suitable for mass examination.

The methods of Giemsa, Wright, Romanowsky, Leishman and Field can be substituted by the simple and inexpensive procedures of Saye or of Chetnikoff for the staining of blood parasites and also of intestinal protozoa. The technique of Saye was originally devised for the staining of blood smears for differential counts. It is suitable for thin smears without alteration. When it is applied to thick smears, a preliminary dehemoglobinization has to be carried out. The usual formol-acetic acid mixture with subsequent neutralization proved satisfactory for this purpose. If Saye's method is used for smears from feces, fixation in Carnoy's or in Bouin's fluid has to precede the staining.

12. *The Occurrence of the Pentastomid, Porocephalus crotali Humbolt, in Rattlesnakes of Southwestern Oklahoma*. J. TEAGUE SELF, University of Oklahoma.

A collection of Pentastomids from the lungs and tracheae of *Crotalis a. atrox* Baird & Baird, collected on the Wichita Mountains Wildlife Refuge, has been furnished the author by Frank B.

McMurry. These have been determined as *Porocephalus crotali* (Humbolt 1808) Humbolt 1811, previously unreported in this region of the United States. Infections are common and heavy, as many as 158 specimens having been collected from a single host. The prevalence of *Porocephalus crotali* in this area suggests a wide distribution in the southwest.

13. *Paramphistomes of North American Domestic Ruminants*. EMMETT W. PRICE AND ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

Until recent years it was generally believed that the rumen flukes of cattle in the United States belonged to the species *Paramphistomum cervi* (Zeder). Krull (1932, 1934) and Bennett (1936) reported on the life history of an amphistome which they regarded as *Cotylophoron cotylophorum* (Fischöeder) and since then this species has been regarded as the common rumen fluke of American livestock. During the past year the writers have studied the amphistome material available in the U. S. National Museum and find the following species to be present in American ruminants: *Paramphistomum cervi* (Zeder) in cattle, relatively rare; *P. liorchis* Fischöeder in cattle and deer in Florida and Louisiana, common in deer; *Cotylophoron* sp., possibly *C. cotylophorum*, in sheep in New York State, rare (one record); and *Paramphistomum microbothrioides*, n. sp., in cattle, common and widely distributed. *P. microbothrioides* is closely related to *P. microbothrium* Fischöeder, but differs from it in several respects, particularly in the number and distribution of the dorsal external acetabular muscle fibers. The dorsal external 2 fibers (Näsmark's nomenclature) consist of about 6 to 9 irregularly spaced units in *P. microbothrioides* in contrast to about 25 regularly spaced units in *P. microbothrium*. The species referred to as *Cotylophoron cotylophorum* by Krull (1932, J. Parasitol. 19: 165-166; 1934, 20: 173-180) and by Bennett (1936, Ill. Biol. Monogr. 14(4)) is identical with *P. microbothrioides*.

14. *Trypanosomes in North American Amphibians*. ROSS F. NIGRELLI, New York Zoological Society.

The following trypanosomes have been reported from North American amphibians: *T. diemyctyli* Tobey (1906) in *Triturus viridescens*; *T. clamatae* Stebbins (1907) in *Rana clamitans*; *T. parvum* Kudo (1922) in *Rana clamitans*; *T. cryptobranchi* Roudabush and Coatney (1937) in *Cryptobranchus alleganiensis*. Recently, Fantham, Porter and Robertson (1942) reported as new *T. lavalis*, *T. gaumontis* and *T. montrealis* in *Bufo americanus* from Canada. Due to the extreme polymorphism demonstrated by amphibian trypanosomes, it is very difficult to determine the validity of these species. In addition, *Trypanosoma rotatorium* (Mayer, 1843) has been reported in *Rana clamitans*, *R. catesbiana*, *R. sphenoccephala*, *R. pipiens*, *Bufo fowleri*, *Hyla crucifer*, and *Pseudacris brimleyi*. *Trypanosoma inopinatum* Ed. and Ét. Sergent (1904) was reported in *R. pipiens*. In the present survey the following amphibians were found infected: *Triturus viridescens*, *Acris gryllus*, *Hyla versicolor*, *H. crucifer*, *H. andersoni*, *R. pipiens*, *R. clamitans* and *R. catesbiana*. The trypanosome found in *Acris* is considered new and the name *Trypanosoma grylli* is proposed. Twenty-four individuals examined were found to be 100 per cent infected. The parasites, which are present in large numbers, approach the size reported for human trypanosomes and resemble them in many respects. They measure from 15.7 to 20 microns in length (av. 17.43) and from 4.2 to 6.6 microns in width (av. 5.4). The body is highly metabolic and the cytoplasm shows considerable vacuolation. However, the form taken by these trypanosomes is a constant feature. The posterior end is rounded, while the anterior end tapers slightly. The kinetoplast is well developed, the parabasal body lying a short distance from the posterior end. The undulating membrane is not exceptionally formed, but the free flagellum is comparatively long. The nucleus is always posterior in position, invariably lying close to the kinetoplast.

15. *A Blood Parasite of the Turkey*. E. MCNEIL AND W. R. HINSHAW, University of California.

This paper reports the finding of an intra-erythrocytic blood parasite from 10-week-old turkey poults. It varies in diameter from 0.5  $\mu$  to 2.0  $\mu$  with an average of 1.0  $\mu$ , and is situated approximately halfway between the nucleus and the edge of the cell. It is usually almost round, but may be oval or pear-shaped. Usually there is only one parasite within a cell, and never more than two. It has been found in cells in the liver and in the peripheral blood. The blood picture shows a slight anemia with some poikilocytosis. At autopsy the principal findings are flabby hearts, congestion of the intestine with diarrhea, and failure of the blood to clot normally. This parasite probably belongs in the Babesiidae, and rather closely resembles *Sauroplasma thomasi* Du Toit.

16. *The Continuous Culture of Pure Clones of Dero limosa Infected with Adelina deronis*. THEODORE S. HAUSCHKA, Lankenau Hospital Research Institute.

Uninfected clones of the aquatic oligochaete *Dero limosa*, derived by fission from single individuals, may be grown continuously on scalded lettuce with the addition of eight B-complex

vitamins (Hauschka, 1944, Growth 8: March issue, in press). Worms heavily infected with the coccidian *Adelina deronis* (Hauschka, 1943, J. Morph. 73: 529-582) can likewise be cultured on the above medium. The marked transparency of the host facilitates determination of the extent of infection in vivo. Close correlation was found between severity of infection and fission frequency which is relatively constant in parasite-free controls. For instance: Eight uninfected clones, each derived from a single animal, showed the following increase after four weeks: 5, 6, 7, 7, 7, 8, 8 (average 6.75; range 5-8). Eight infected lines, also derived from single worms, after four weeks contained: 1, 2, 2, 4, 5, 6, 7, 8 animals (average 4.37; range 1-8). With proper care, i.e., change of culture medium and dish twice a week, even the most heavily parasitized clones will survive for indefinite periods. At present (June 1944), five infected pure-line clones have been growing for nine months and show no decline. The cultures contain infections of varying intensities with no indication of acquired immunity. The nine-month-period of culture represents 27 generations of the host and approximately 15 generations (life cycle 18 days) of the parasite.

17. *A Case of Trichostrongylus sp. with Notes on the Identification of Ova.* H. TSUCHIYA AND HELEN RELLER, Washington University.

A patient, 56 years of age, Greek, entered Barnes Hospital with complaint of intermittent pain in epigastrium and upper right quadrant, chronic constipation, dyspnoea, productive cough, headache, ringing of left ear and a loss of 28 pounds in one year. Stool examination revealed a few ova of *Trichostrongylus sp.* (by Willis flotation method) and cysts of *Giardia lamblia*. Blood counts: red cells, 5,120,000; white cells, 6,400; haemoglobin, 86 per cent; no eosinophiles. He died shortly after admission. Clinical diagnosis: generalized abdominal carcinomatosis, senile emphysema and syphilis of undiagnosed site. Since autopsy was not performed, the species of *Trichostrongylus* remains undetermined. As compared with hookworm ova, they are much longer and slightly wider, measuring 81 to 97 microns in length by 40 to 51 microns in width, averaging 86 by 43 microns, and have elongated oval-shape with transparent shell membrane which is thicker and more greenish and lustrous. While the ends of the ova are more pointed than those of hookworm, in a majority of them one end is somewhat pointed while the other is rounded, resembling somewhat the shape of a grape. The pointed end appears slightly thicker than the non-pointed end. Ova in freshly voided stool present 16-24 morula stage of embryonation which, together with the characteristics described above, differentiate them from hookworm ova.

18. *The Production of Anemia in Lambs by Hookworms, Bunostomum trigonocephalum.* JOHN T. LUCKER AND ELEANOR M. NEUMAYER, U. S. Bureau of Animal Industry.

A *Bunostomum*-free lamb receiving percutaneously a dose of 50,000 hookworm larvae, when 4½ months old, became heavily infected and markedly anemic. Another lamb, that had failed to become appreciably infected following oral administration of 5,000 larvae, also became markedly anemic following application to its skin, when 8½ months old, of 11 doses totalling 112,000 larvae. The lowest observed levels in cell-volume percentages, hemoglobin and erythrocytes occurred 11 to 14 weeks after infection and were about one-half normal values. At necropsy, 17 to 19 weeks after infection, these lambs harbored, respectively, 1,500 and 2,000 hookworms. A third lamb, which had failed to become infected following oral administration of larvae, received percutaneously, when 8½ months old, 580,000 larvae in two applications. A light infection characterized by moderate anemia resulted. Blood appeared in the feces of these lambs within 25 days after infection. A fourth lamb developing a light infection but no anemia following application to its skin of 5,000 larvae in 10 equal weekly doses, remained unaffected when 560,000 larvae were applied to its skin in 11 doses 4 months later. At necropsy these lambs harbored respectively 80 and 190 hookworms. These four lambs were fed alfalfa hay and grain. In an uncompleted experiment involving two identically dosed groups of younger lambs, one receiving this diet and the second timothy hay, a lamb on the latter diet developed an extreme anemia terminating fatally about 7 weeks after infection, before eggs appeared in its feces.

19. *Comparative Growth Rates of Amoebae and Bacteria in Cultures of Endamoeba histolytica and Organism t.* CHAS. W. REES AND LUCY V. REARDON, National Institute of Health.

*Endamoeba histolytica* and a single species of bacteria, organism *t*, were grown in Florence flasks of 250 ml capacity in diphasic media of coagulated whole egg, coagulated egg white, and liver infusion agar, respectively; also in a number of wholly liquid media. The basic liquid was Locke's solution without serum supplemented with rice flour, buffered with egg shell, and with and without other enrichment. Excepting whole egg medium, enrichment with 8 water-soluble vitamins and cholesterol was used. The liquid media contained commercial peptones or papain digests of peanut, soy-bean, cottonseed, rice, coagulated whole egg or egg white, and dried hog liver. Amoeba growth was determined by haemocytometer counts and bacteria growth by gas

measurements. At 72 hours of cultivation each flask of diphasic whole egg and enriched egg white medium produced up to 3,000,000 amoebae and 100 ml of gas. Liver infusion agar produced few amoebae and much gas. Without vitamin-cholesterol enrichment, egg white medium gave poor growth of amoebae and bacteria. Without dextrose all media gave poor growth of both organisms. In several cases digest media produced about 1,000,000 amoebae but poor growth in other cases. With several exceptions bacteria growth in digest media was as high at 24 hours as in good amoebae producing media at 72 hours. Twelve commercial peptones with vitamins and cholesterol gave poor growth of amoebae and excellent growth of bacteria.

In culture with organism *t*, *E. histolytica* utilizes cholesterol, rice flour, undermined ingredients of egg white, and vitamins. Organism *t* utilizes dextrose, vitamins, and nitrogenous materials occurring in all the media that were tested.

20. *Inoculations of Trichomonas foetus (Protozoa) in Heifers.* BANNER BILL MORGAN, University of Wisconsin.

Nine virgin heifers, all about 12 months of age, were injected with *Trichomonas foetus*. Five of the heifers were inoculated intramuscularly along the mid-neck with 5 ml of living, washed, bacteria-free trichomonads approximately twice per week for a period of 3 months. The suspension began with 5 million organisms per ml and ended with 50 million. Two heifers were injected intramuscularly with formalin-killed trichomonads. The remaining 2 heifers were injected intravenously with living trichomonads. Each animal received 17 injections. In the sera of these heifers immobilization of the trichomonads was observed in dilutions ranging from 1:4 to 1:128. One animal which had been injected intravenously died of an intussusception as a result of an anaphylactic shock. At the first heat period after the last injection each cow was artificially inseminated with fresh normal semen. Twenty-four hours later all cows were inoculated in each uterine horn with 10 ml of motile *T. foetus* containing 50 million organisms per ml. Nine noninjected, virgin heifers, all about 12 months of age, used for controls were inseminated and inoculated in a similar fashion. Four of the heifers conceived on the first service and calved normally. Two conceived on the second insemination, 2 on the third, and all gave birth to normal calves. The remaining heifer developed a mild case of rickets and was given a short breeding rest, but this animal conceived and calved normally after the fourth insemination. All nine controls developed varying degrees of vaginitis, endometritis and pyometra. One heifer produced a normal calf but only after a severe case of pyometra. Trichomonads were isolated at various intervals from the controls; however, in only one injected heifer could the organisms be found.

21. *The action of Negro and White Sera on Infective Hookworm (Necator americanus) Larvae.* GILBERT F. OTTO, EVELYN ALLEN AND STERLING BRACKETT, Johns Hopkins University.

The precipitin reaction resulting from living hookworm larvae in human sera had no demonstrable relation to the infection status of the individuals from which the sera were obtained. Less than 30 per cent of the whites from the endemic area had sera which reacted strongly whereas 90 per cent of the negroes had reacting sera of which about two-thirds reacted strongly. Studies in progress suggest that negroes outside the endemic area may have strongly reacting sera.

22. *Tests to Determine the Ability of Species of Domestic Mosquitoes to Transmit Wuchereria bancrofti.* WALTER L. NEWTON AND WILLARD H. WRIGHT, National Institute of Health.

Because of the possibility of the reestablishment of filariasis in the continental United States through the return of infected individuals from endemic areas abroad, tests are being carried out to determine the domestic mosquito vectors of *Wuchereria bancrofti* (periodic form). To date, a total of 951 dissections has been made of 7 species of mosquitoes, 640 of which lived 9½ days or longer after feeding on an infected individual. In a control test with a relatively small number of *Culex quinquefasciatus*, all specimens developed infective larvae, thus confirming the findings of Francis (1919) that this species is an efficient vector in the continental United States. Of the 6 other species, the respective percentages which developed infective or potentially infective microfilariae were, as follows: *Anopheles albimanus* 67; *Psorophora confinis* 12; *Culex nigripalpus* 6.8; *Aedes aegypti* 4.9; *Aedes sollicitans* 0; and *Aedes taeniorhynchus* 0. These results indicate that *A. albimanus*, *P. confinis*, *C. nigripalpus*, and *A. aegypti* are potential vectors. Apparently, the salt marsh species, *A. sollicitans* and *A. taeniorhynchus* would be incapable of transmitting infection under the conditions prevailing during these experiments.

23. *Agglutination of Microfilariae from Litomosoides carinii of the Cotton Rat by Normal Serums.* JAMES T. CULBERTSON AND HARRY M. ROSE, Columbia University.

When normal serum from man or from certain laboratory animals (e.g., rabbit) was added to a suspension of embryos obtained from *Litomosoides carinii* of the cotton rat, the embryos were



agglutinated. The tests were carried out on microscope slides. One drop of undiluted, inactivated serum was added to one drop of a heavy suspension of embryos on the slide, and the slide rotated to effect mixing of the fluids. The mixtures were examined for agglutination after 10 minutes under seven times magnification. Among 55 unselected Wassermann laboratory blood serum samples, 39 (70.9%) were positive. The serums of albino rats and of cotton rats (some of the latter animals infected with *Litomosoides carinii* and others not infected) did not agglutinate the embryos.

24. *Cross Reactions between Polysaccharides from Various Animal Parasites.* JOSÉ OLIVER-GONZÁLEZ, School of Tropical Medicine, Puerto Rico.

A polysaccharide fraction has been isolated from the following helminths: *Ascaris lumbricoides*, *Trichinella spiralis*, *Necator americanus*, *Schistosoma mansoni*, *Fasciola hepatica* and *Taenia saginata*, using the method described by Melcher and Campbell (1942). The purified polysaccharide fractions seem to have a very close immunological relationship to each other. Each of the fractions, when added in suitable concentrations to human serums, inhibit the  $\alpha$  and  $\beta$  isoagglutinins present in the serums. The serums of rabbits immunized with any of the polysaccharide fractions react to about the same precipitin titer with the homologous and heterologous polysaccharide test antigens. The following table is illustrative:

Test antigen	Antiserum
Polysaccharide from:	Rabbit immunized with polysaccharide from <i>Taenia saginata</i>
	Precipitin titers
<i>A. lumbricoides</i>	12,800
<i>T. spiralis</i>	12,800
<i>N. americanus</i>	12,800
<i>F. hepatica</i>	6,400
<i>S. mansoni</i>	6,400
<i>T. saginata</i>	12,800

The presence of such a common antigenic substance in all of the above-mentioned helminths may be of importance in relation to cross reactions which might exist between these organisms during infection or during artificial immunization. Investigations on this particular point as well as on the relations between the polysaccharide fractions and the human isoagglutinogens are "in progress.

25. *The Effects of Host Starvation on Worm Elimination and Glycogen Depletion with the Nematode Ascaridia galli.* W. MALCOLM REID, Monmouth College.

The mature fowl nematode *A. galli*, like the cestode *Raillietina cesticillus* (J. Parasitol. 28: 319-340), is eliminated from the host by short starvation periods. Although cestode strobilae were passed in 24 to 48 hours, the nematode required 48 to 96 hours of starvation. Ten out of 14 fowls lost all parasites at the end of 96 hours, while the remaining four contained but 1, 2, 5, and 11 worms. Ten unstarved controls contained from five to 45 worms at autopsy, the average infection being 22.7 worms. Thirteen of the experimental group eliminated one to 19 worms with an average of 7.2 parasites per fowl, while four controls lost single worms during the experiments. It is probable that many dead parasites were not recovered from starved hosts since the worms in the droppings showed various stages of degeneration.

The glycogen content of female worms removed from unstarved chickens averaged 4.63 per cent, while after 24 hours of host starvation this level was reduced to 1.35 per cent and after 48 hours to 0.49 per cent. Males under similar conditions showed an average of 3.60, 1.36 and 0.46 per cent respectively. Extreme glycogen depletion is correlated with and possibly responsible for the elimination of these two species of parasites. This sensitivity of parasites to feeding conditions of the host needs to be considered in planning for, carrying out, and interpreting experiments.

26. *Substitution of Soybean Oil Meal for Animal Protein in Developing Resistance of Animals to Parasitism.* J. E. ACKERT, DOROTHY S. BRANSON, D. J. AMEEL AND B. B. RIEDEL, Kansas State College.

In previous experiments it was found that when peanut meal was used as a protein supplement to a basal cereal ration the resistance of chickens to the viability and growth of the fowl

ascarid *Ascaridia galli* was lowered in comparison with that of fowls whose basal cereal ration was supplemented with meat scraps or meat scraps and milk.

Similar tests on more than 400 growing chickens parasitized with *A. galli* have been made with a soybean oil meal supplement in place of the peanut meal. Other groups of chicks of the same age were given meat scraps, or meat scraps and powdered skim milk as supplements to the otherwise adequate cereal basal ration.

The soybean oil meal group, instead of having more worms and longer ones as in the case of the peanut meal groups, had *A. galli* in approximately the same average number and length as those in the meat scraps, or in the meat scraps and milk supplement group. The trend was toward higher resistance in the soybean oil meal group, but the differences were not significant. The explanation for the success of this all-plant ration in producing host resistance probably lies in the high percentage of protein-digestibility of soybean oil meal in growing fowls.

27. *Studies on Effects of Helminths on Growing Chickens.* J. E. ACKERT AND C. L. WISSEMAN, JR., Kansas State College.

Tests were made of effects on chickens of smaller numbers of the nematode *Ascaridia galli* and of the cestode *Raillietina cesticillus*, respectively. Of 30 chicks maintained on an adequate diet, 15 were parasitized with 100 embryonated eggs of *A. galli* and the balance kept as controls. Comparisons of weights, haemoglobin percentages, and blood sugar levels showed no significant differences between the parasitized and control groups. Post-mortem examinations showed infections of from one to 46 *A. galli*, with an average of 17.9 worms per chicken. Lack of conclusive evidence of harmful effects of the worms is attributed to an improved ration for the chickens and to the variability of the blood sugar levels of the chickens.

Tests for effects of moderate infections of tapeworms on chickens were carried out on 44 young chickens, half of which were parasitized with the fowl tapeworm *Raillietina cesticillus*, and the balance kept as controls. All chickens were kept on the same adequate ration. Weekly weight records were taken and blood sugar levels and haemoglobin percentages were taken after two weeks and eight weeks of parasitism. Differential white cell counts were made four weeks after infection. Although 21 of the birds had 16 or more tapeworms and five had in excess of 100, no constant differences in the parasitized and controlled groups were found in growth of chickens, blood sugar level, haemoglobin estimate or differential white cell counts. These results indicate that if all of the ingredients required by growing chickens are supplied, the young fowls can tolerate considerable numbers of tapeworms without serious effects.

28. *The Effects of Nematode Infections During the Prepatent Period on the Calf.* ROY L. MAYHEW, Louisiana State University.

Weights of six calves kept under parasite-free conditions were found to have their rate of gain very much reduced when inoculated with pure suspensions of stomach worm (*Haemonchus contortus*) larvae and mixed suspensions of stomach worm and nodular worm larvae (*Oesophagostomum radiatum*). As the larvae reached maturity, and eggs appeared in the manure, the regular rate of gain was resumed and maintained.

The deaths of six animals inoculated with mixed suspensions of stomach and nodular worms have resulted during the larval period of these parasites. These animals were raised parasite-free and the results of inoculation were confirmed at post mortem by marked inflammation in the stomach and small and large intestines and by the recovery of the parasites.

The severe anemia which has been found to develop during the prepatent period may lower the general resistance of the calf to the other diseases.

29. *Alcoholism in Mice and Its Effect on Natural and Acquired Resistance to Hymenolepis nana var. fraterna.* JOHN E. LARSH, JR., University of North Carolina.

Young mice have been tested to determine the effect of alcoholic intoxication on natural and acquired resistance to *H. nana var. fraterna*. The first tests were made after three weeks daily treatment with 0.4 cc of 35 per cent alcohol by mouth. Following proportionate egg doses, cysticercoid counts showed about twice the number of parasites in the alcoholic group as in controls of the same age. Animals reacted differently which were immunized by infection several days before beginning the alcohol. Very few cysticercoids were observed in either the alcoholic or the non-alcoholic group, whereas there were typical numbers in a second non-alcoholic group which was not infected previously. Tests were made to see what the effect would be of intoxication produced just one hour prior to initial infection. The controls had typical numbers of parasites, but exceedingly few were observed in mice of the intoxicated group. During intoxication the rectal temperature falls sharply from 37° C to 33° C and remains low for several hours. Inasmuch as this may have had an effect on the hatching of the eggs, tests were designed to control it. After intoxication the mice were placed in a large incubator at 37.5° C and within an hour when the temperature returned to normal they were infected. About the same number of

parasites was observed in both the alcoholic and non-alcoholic group. Thus alcohol appears to interfere with the mechanism of natural resistance but not the immune state. How the drug exerts its deleterious effect is unknown but studies on this are in progress.

30. *Critical Tests with a Hexachlorethane-bentonite-water Suspension as a Fasciolicide in Cattle.* O. WILFORD OLSEN, U. S. Bureau of Animal Industry.

Post-mortem examinations on 34 grown range cattle in the Gulf Coast region of Texas, known to have been infected with *Fasciola hepatica* at the time of treatment, showed that a single dose of hexachlorethane suspended in bentonite and water (Olsen, 1943, J. Am. Vet. Med. Assoc. 102: 433-436) was highly efficacious against liver flukes, especially against the adult worm.

A total of 74 young flukes and 17 adult flukes were found in the livers of the 34 treated animals, 10 to 50 days after treatment. The mean number of young flukes per treated animal was 2.16, the range being 1 to 13 each, in 17 cows; the mean number of adult flukes was 0.5 per animal, the range being 1 to 5 each, in 7 cows. The mean number of flukes of all sizes for the 34 animals was 2.67, with a range of 1 to 13 each, in 21 cows.

Seven control cows, slaughtered from the same herd on the same date with 13 of the experimental cows, showed a total of 181 young flukes and 100 adult flukes. The average number of young flukes was 25.8, with a range of 55 to 126, in 2 animals; the average number of adult flukes was 14.2, with a range of 6 to 44 each, in 5 cows. The average number of flukes of all sizes for each of the control cows was 40.1.

All except extremely debilitated cattle tolerate a dose of 100 grams of hexachlorethane in suspension (200 ml) very well. Cattle in average condition have received twice this dose without showing untoward effects.

31. *Common Names of Helminths.* DONALD B. McMULLEN, University of Oklahoma; H. L. VAN VOLKENBERG, Texas Agricultural and Mechanical College; and PAUL D. HARWOOD, Drs. Hess and Clark Laboratories, Inc.

Helminthologists, who are in contact with governmental extension services, or with commercial organizations, are frequently perplexed by a demand for common names of parasitic worms because there is no established or uniform usage in many instances. Accordingly, a committee of this society was charged with preparing a tentative list. Textbooks by American authors, bulletins from the U. S. Department of Agriculture and from the various states were searched for common names already in use. Established names should be accepted with as little change as may be necessary to prevent duplication. However, a variety of worms have been called simply stomach worms by various authors. In the publications where these names appear, the meaning is clear, but for purposes of a list of common names, other delimiting adjectives or phrases may be necessary. Thus *Hyostromylus rubidus* may be called the red stomach worm of swine. Also similar names should be used for related worms. In a very few instances new or highly modified names may be necessary. However, the committee believes that a list of common names to be useful must be a cooperative project. Therefore, the names proposed by the committee are regarded merely as a basis for discussion. Opinions have been, or will be, sought from many parasitologists, and on this basis a preferred name will be proposed. Therefore, a common name is sought which is brief, descriptive, and preferred by the majority of scientists.

32. *A Reaction of Rat Serum to Embryonated Eggs of Trichosomoides crassicauda.* VIVIAN S. SMITH, University of Illinois.

Serum from albino rats infected with *Trichosomoides crassicauda* was used in the preparation of vaseline-sealed coverglass cultures of *T. crassicauda* eggs. Cultures were prepared using eggs repeatedly washed in sterile saline after being teased from gravid female worms.

After incubation at 37° C for eight hours or longer, a flocculum was observed around numerous embryonated eggs. Serum of rats which had been repeatedly infected showed earlier appearance of flocculum, more numerous eggs affected, and greater amounts around individual eggs, as compared with rats which had been infected only once. Control cultures using serum of animals which had been kept parasite-free showed no reaction to *T. crassicauda* eggs. Although several larvae generally hatched out in each culture, and were observed to survive as long as 11 days, no flocculum appeared around them in any instance.

A satisfactory explanation for the serum reaction to embryonated eggs rather than to larvae has not been worked out at present. However, the reaction is thought to be of antigen-antibody nature.

33. *Studies on the Relationship of Trichosomoides crassicauda Infection in Rats to Mucoid Calculi of the Urinary Bladder.* VIVIAN S. SMITH, University of Illinois.

An unusual type of calculus is commonly found in the urinary bladders of wild and laboratory rats. Of 32 instances among 370 wild rats, 27 calculi were associated with the presence of

*Trichosomoides crassicauda*. Among albino laboratory rats used for *T. crassicauda* experiments, however, two of nine vesical calculi were in control animals which had been raised parasite-free. Although mice have not been reported to harbor this helminth parasite, two typical calculi were found in albino males. A striking feature is that all of the above-mentioned instances were in male animals except for two cases in female wild rats.

Vesical calculi of the type described are irregular in shape, whitish in color, and of a soft gum-rubber consistency. Sections frequently showed worms embedded in the acellular matrix. Dried ground calculus material gave a strongly positive biuret test. Use of mucicarmine stain on sections indicated that the masses were composed largely of mucin.

*T. crassicauda* is not believed to initiate calculus formation, but may possibly serve as a secondary factor. Preponderance of mucoid calculi in male animals is not definitely explained. A hypothesis that seems feasible is that a back-flow of mucoïd secretion from the seminal vesicles and prostate into the urinary bladder might occur under certain conditions. Presence of worms would further favor calculus formation by inducing excess secretion of mucus through irritation of the bladder epithelium. Experimental operative insertion of various foreign substances into the bladder failed to induce formation of mucoid calculi.

34. *The Possibility of Eliminating the Schistosome Snail, Australorbis glabratus, from Canals by Control of Water Flow.* GEORGE W. LUTTERMOSER, Oficina Cooperativa Inter-Americana de Salud Publica (Venezuela).

In order to determine which water velocities the snail carrier of *Schistosoma mansoni*, *Australorbis glabratus*, could resist, the following canals were used: a wooden canal 5 meters long by 15 cm deep by 21 cm wide and a semi-circular concrete canal with a radius of 7.6 cm and a length of 5 meters. Several adult snails were placed in the lower end of the canals and a stream of water entered the higher end. The flow of water and the slope of the canals were fixed and determined for each test.

In the 14 experiments with the wooden canal, it was found that the snails were able to maintain themselves for more than three hours and advance upstream 26 and 52 cm per hour in the water at velocities of 0.06 and 0.15 meter per second. When the water velocity was increased to 0.25 meter per second, the snails were only able to remain in the canal for three hours and they advanced upstream 1 to 3½ cm per hour. In velocities of 0.32 or 0.33 meter of water per second, the *A. glabratus* made no progress upstream and were all washed downstream within an hour. Flows of 0.38 to 0.42 meter per second prevented the snails from going upstream and they were all carried away in 10 to 30 minutes. The results of 10 experiments with the concrete canal were similar to those with the wooden canal.

It is concluded that relatively low water velocities should eliminate *A. glabratus* from well-constructed canals in the field. A test of this possibility is being made.

35. *The Parasites of the Plathodontidae (Amphibia—Caudata) III.* A. C. WALTON, Knox College.

Annotated lists of the parasites of the Plathodontidae indicate the following hosts and parasites: (a) *Eurycea tynerensis* (U.S.A.)—*Acanthocephalus vanclaei* (Acanthocephala). (b) *Hydromantes genei genei* (S. Europe)—*Chondrostagon haematicum*, and *Pirhaemocyton tarentolae* (Protozoa); and *Batrachobdella algira* (Hirudinea). (c) *Pseudotriton montanus* (U.S.A.)—*Physaloptera* larvae of Morgan, 1941 (Nematoda); *Allocreadium pseudotritoni*, *Gorgoderina amplicava*, and *Gorgoderina bilobata* (Trematoda); *Haptophrya michiganensis*, *Proteromonas longifila*, and *Trichomonas augusta* (Protozoa); and *Hannemania dunni* (Acarina). (d) *Pseudotriton ruber* (U.S.A.)—*Oxyuris* dubia, and (?) *Oxysomatium brevicaudatum* (Nematoda); *Allocreadium pseudotritoni*, *Brachycoelium salamandrae*, *Gorgoderina amplicava*, *G. cygnoides*, *Gorgoderina bilobata*, and "Metacercariae" of Rankin, 1937 (Trematoda); larval *Crepidobothrium* sp? of Rankin, 1937, *Ophiotaenia cryptobranchi*, and Proteocephalid cysts of Mann, 1932, and of Rankin, 1937 (Cestoda); and *Cryptobia borrelli*, *Cytamoeba bacterifera*, *Eutrichomastix batrachorum*, *Hexamastix batrachorum*, *Hexamita intestinalis*, *Karotomomorpha swezyi*, *Proteromonas longifila*, and *Trichomonas augusta* (Protozoa).

36. *The Parasites of the Plathodontidae (Amphibia—Caudata) IV.* A. C. WALTON, Knox College.

Annotated lists of the parasites of the Plathodontidae indicate the following hosts and parasites: (a) *Plethodon cinereus* (*P. erythronotus*) (Canada)—*Angiostoma plethodontis*, and "*Oxyuris*" *magnavulvaris* (Nematoda); *Brachycoelium salamandrae*, and *B. sp?* of Rankin, 1938 (Trematoda); larval *Crepidobothrium* sp? of Rankin, 1937 (Cestoda); and *Cryptobia borrelli*, *Cytamoeba bacterifera*, *Eutrichomastix batrachorum*, *Haptophrya michiganensis*, *Hexamastix batrachorum*, *Hexamita batrachorum*, *H. intestinalis*, *Karotomomorpha swezyi*, *Proteromonas*



*longifila*, and *Trichomonas augusta* (Protozoa). (b) *Plethodon glutinosus* (U.S.A.)—"Oxyuris" *magnavulvaris*, larval *Physaloptera* sp? of Walton, 1935, and of Rieber, Byrd, and Parker, 1940 (Nematoda); *Brachycoelium salamandrae*, and *B. sp?* of Rankin, 1938 (Trematoda); Proteocephalid cysts of Mann, 1932, and of Rankin, 1937 (Cestoda); *Acanthocephalus acutulus* (Acanthocephala); *Cryptobia borrelli*, *Cytamoeba bacterifera*, *Eutrichomastix batrachorum*, *Haptophrya gigantea*, *H. michiganensis*, *Hexamastix batrachorum*, *Hexamita batrachorum*, *H. intestinalis*, *Karotomorpha svezyi*, *Proteromonas longifila*, and *Trichomonas augusta* (Protozoa); and *Hannemania dunni* (Acarina). (c) *Plethodon metcalfi* (U.S.A.)—*Brachycoelium salamandrae* (Trematoda); larval *Crepidobothrium* sp? of Rankin, 1937, and *Ophiotania cryptobranchi* (Cestoda); and *Cryptobia borrelli*, *Eutrichomastix batrachorum*, *Hexamastix batrachorum*, *Hexamita intestinalis*, *Karotomorpha svezyi*, *Proteromonas longifila*, and *Trichomonas augusta* (Protozoa). (d) *Plethodon yonahlossee* (U.S.A.)—"Oxyuris" *magnavulvaris* (Nematoda); *Brachycoelium salamandrae* (Trematoda); and *Cytamoeba bacterifera*, *Eutrichomastix batrachorum*, *Karotomorpha svezyi*, *Proteromonas longifila*, and *Trichomonas augusta* (Protozoa).

37. *The Parasites of the Proteidae and the Sirenidae (Amphibia—Caudata)*. A. C. WALTON, Knox College.

Annotated lists of the parasites of the Proteidae indicate the following hosts and parasites: (a) *Necturus maculosus* (U.S.A.)—larval *Eustrongylides ignotus*, and *Oswaldocruzia subauricularis* (Nematoda); ? *Crepidostomum cooperi*, ? *C. cornutum*, ? *C. farionis* (also in Canada), *Gorgoderina schistorchis*, *Monocaeum baryurum* (Canada), *Neochasmus umbellus*, *Sphyranura oligorchis*, *S. osleri*, and *Telorchis necturi* (Trematoda); ? *Nematotaenia dispar*, and *Ophiotania lönnbergii* (Cestoda); *Trichodina pediculus* (Protozoa); and *Simpsonichoncha ambigua glochidia* (Pelecypoda). (b) *Proteus anguinus* (Europe)—*Agamonema proteis-anguini*, and *Hedruris androphora* (Nematoda); and *Chloromyxum protei* (Protozoa). Study of the Sirenidae indicate the following host and parasites: (a) *Siren lacertina* (U.S.A.)—*Multicaecum tenuicolle*, *Oxysomatium americana*, and *Spirooura catesbiana* (Nematoda); and larval *Cephalogonimus amphiumae*, larval *Clinostomum marginatum*, *Diplostomulum sirenis*, *D. trituri*, *D. sp?* of Manter, 1938, larval, *Distoma sirenis lacertinae*, *Gorgodera minima*, larval *Monostomulum asperum*, and *Telorchis sirenis* (Trematoda).

38. *Effect of Skim Milk on Infections of Ascarids, Whipworms, and Nodular Worms in Swine*. L. A. SPINDLER AND HARRY E. ZIMMERMAN, JR., U. S. Bureau of Animal Industry.

Spindler, Zimmerman and Hill (1944, Proc. Helm. Soc. Wash. 11(1): 9-12) reported that pigs fed only fluid skim milk for 3 to 5 days expelled during that time the vast majority of their whipworms and nodular worms and smaller numbers of ascarids. Subsequently a test was carried out to ascertain whether by feeding skim milk, infections of the parasites named could be kept at a low level in pigs constantly exposed to infection. Nine 12-weeks-old, littermate pigs, free of worms, except *Strongyloides*, were divided into 3 equal groups and kept on contaminated ground. Pigs of group 1 were fed as much skim milk as they would drink each evening; group 2 was fed skim milk as the sole component of the diet for 3 days at intervals of two weeks; in both cases the quantity administered was sufficient to produce copious purging. When milk was not being fed, a balanced grain ration was fed on the ground. Group 3 was fed only grain on the ground and therefore served as a control.

At post mortem after 98 days on test, worm infections found in the control pigs (group 3) were as follows: Ascarids, 55, 116, 88; whipworms, 150, 97, 221; nodular worms, 115, 225, 153. None of the group 2 pigs harbored any of the parasites named. Two of the group 1 pigs were uninfected, and the third harbored 20 whipworms.

Results of this investigation indicate that frequent administration to pigs of fluid skim milk in quantities sufficient to purge will protect the animals against severe infections of the helminths named.

39. *An in vitro Method for the Bio-Assay of Chemotherapeutic Agents in Filariasis*. HARRY M. ROSE, JAMES T. CULBERTSON AND ELEANORA MOLLOY, Columbia University.

A satisfactory *in vitro* method has been devised for the biological assay of chemotherapeutic agents in filariasis, using *Litomosoides carinii* as the test organism. This parasite is found naturally to infect a considerable proportion of Florida cotton rats, the adult worms invariably residing in the pleural space, while unensheathed, non-periodic microfilariae occur in the peripheral blood of the animals.

The adult parasites are removed from the thoracic cavity with aseptic precautions, and suspensions of microfilariae are obtained by washing out the pleural space with sterile physiological saline. Adults and microfilariae are then transferred to 50 ml. Erlenmeyer flasks containing 10 ml. of sterile physiological serum-salt solution (Simms) prepared as follows:

Solution A	Grams per liter
NaCl	160.0
KCl	4.0
CaCl <sub>2</sub> -2H <sub>2</sub> O	0.88
MgCl <sub>2</sub> -6H <sub>2</sub> O	4.06
Solution B	
NaH <sub>2</sub> PO <sub>4</sub> -H <sub>2</sub> O	2.2
Na <sub>2</sub> HPO <sub>4</sub>	18.4
NaHCO <sub>3</sub>	1.3
Dextrose	20.0
Phenol red	0.2

Dilute one volume of Solution A with 18 volumes of distilled water and autoclave. Sterilize Solution B by filtration through a sintered glass filter. For the final solution (pH 7.4) add one volume of B to 19 volumes of diluted A, plus sufficient normal horse serum to make a concentration of 10 per cent.

In this menstrum adult filariae and microfilariae remain viable for at least one week at 37° C. At room temperature the parasites will live from one to three weeks. Drugs to be tested may be added in any desired concentration, and the authors have already demonstrated by this method that the *in vitro* action of neostam and neostibosan closely parallels their effects *in vivo*.

The present method is the first to be described in which drugs may be tested *in vitro* against both adult filariae and microfilariae.

40. *The Trombiculid Mites (Chigger Mites) and Their Relation to Disease.* HENRY E. EWING, U. S. National Museum.  
Presidential Address.

#### SYMPOSIUM ON PARASITOLOGY IN RELATION TO THE WAR

1. *The Wartime Importance of Tropical Diseases.* JAMES S. SIMMONS, Brig. General, Medical Corps, United States Army.  
No abstract.

2. *Malaria and the War.* O. R. MCCOY, Major, Medical Corps, United States Army.  
No abstract.

3. *The Development of Malarial Sporozoites in the Vertebrate Host.* CLAY G. HUFF AND FREDERICK COULSTON, University of Chicago.  
No abstract.

4. *The Present Status of the Filariasis Problem.* HAROLD W. BROWN, Columbia University.

Filarial infections acquired by our armed forces in the Pacific theater of war have stimulated a renewed interest in the problem of filariasis. Although it appears that the only endemic focus of filariasis in Charleston, South Carolina, has disappeared, the possibility of new endemic foci in this country is discussed. Clinical studies have been reported by medical officers in the armed forces which indicate that filarial infections acquired by white adults may differ in their course from infections acquired by natives in childhood. The prolonged prepatent period of infections in adult Americans necessitates a re-evaluation of clinical and laboratory diagnostic methods. Recent studies on the treatment of filariasis indicate that certain antimony compounds possess considerable filaricidal powers. Continuation of these therapeutic studies have led to the discovery of a number of organic compounds of considerable therapeutic promise.

5. *Distribution and Control of the Fevers of the Typhus Group.* R. E. DYER, Assistant Surgeon General; Director, National Institute of Health.

This group of fevers is widespread throughout the world.

Epidemic typhus—Europe, Asia, Africa, South and Central America.

Endemic typhus widely distributed in the tropics and subtropics, but also in temperate climates including the United States.

Scrub typhus—Japan, Southeastern Asia, and the islands of the Southwest Pacific.

Rocky Mountain spotted fever—the Western hemisphere with somewhat similar tick-borne infections around the Mediterranean, East and South Africa, and India.

Control of these diseases depends upon control of the reservoir and of the arthropod vector.

	Reservoirs	Vectors
Epidemic typhus	Man	Body louse
Endemic typhus	Rat	Rat flea
Scrub typhus	Field mice	Mites
Rocky Mountain spotted fever and allied tick-borne diseases	Rodents and domestic animals	Ticks

Measures for control:

Epidemic typhus—Proper isolation of cases with delousing of contacts. Vaccination.

Endemic typhus—Control of the common rat.

Scrub typhus—Personal care, insect repellents.

Rocky Mountain spotted fever—Personal care, repellents, vaccination.

6. *Development of Louse Powders for the Armed Forces.* WALTER E. DOVE, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

Two formulas for the treatment of man with powders for destruction of lice have been developed by E. F. Knipling and R. C. Bushland at Orlando, Florida. Body lice were maintained in a colony, and research subjects were hired for feeding the lice and for conducting the large number of tests with insecticides. Materials showing promise in jar tests were applied to the inside of the sleeves and legs of underwear, and this portion of the underwear was then taped onto the leg or arm of the research subject. Various stages of lice were introduced into these abbreviated garments, where they remained for 24 hours. At the end of this time the number killed by the treatment was determined. In the case of promising lousicides, new lice were added to the garment each day to determine the period of effectiveness.

In this manner a powder was developed known as MYL. This powder killed lice for one to two weeks, and in most instances a single treatment was sufficient. Ten per cent of DDT in pyrophyllite gave protection for two to three weeks and sometimes longer. This material remained in the clothing long enough to kill the young as they hatched from the eggs; it was therefore not necessary to add an ovicide as was done in the MYL treatment.

These powders have been adopted by the armed forces and have been the means of controlling typhus epidemics in the present war.

7. *Parasites as Factors in Production of Meat and Other Animal Products in Wartime.* BENJAMIN SCHWARTZ, U. S. Bureau of Animal Industry.

The war has increased materially the nation's need of meat, leather, wool, mohair, bristles, feathers, catgut sutures, sausage casings, and other animal products. Because they affect adversely the health of animals, zooparasites interfere materially with the production of meat, fiber, leather, and other products of animal origin, the losses sustained in wartime being difficult to overcome through importations.

Among the most serious offenders are cattle grubs (*Hypoderma lineatum* and *H. bovis*) that spoil one out of three hides by puncturing holes in the skin and causing, moreover, considerable loss of meat. Other arthropod parasites also produce skin injuries that render hosts unthrifty, affect the hair coat, and decrease the value of hides for leather.

Gastrointestinal nematodes produce death losses in young animals, and much unthriftiness, especially in growing stock. One species (*Oesophagostomum columbianum*) spoils sheep intestines for catgut sutures and sausage casings. Liver flukes (*Fasciola hepatica* and *Fascioloides magna*), tapeworm cysts (*Taenia tenuicollis*), and fringed tapeworms (*Thysanosoma actinioides*), spoil ruminant livers for food and medicinal preparations, and produce other losses.

Research, begun in peacetime and accelerated under the war impact, lead to practical applications of parasite-control measures, especially medication. The principal medications used to bolster production of meat and other animal products are: (1) rotenone to control arthropod parasites of livestock; (2) phenothiazine to control and eradicate nematodes of ruminants, and (3) a hexachlorethane-bentonite suspension, a product of wartime research in the U. S. Department of Agriculture, to control liver flukes of cattle.

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## EFFECT OF X-IRRADIATION OF RATS UPON THEIR RESISTANCE TO *TRYPANOSOMA LEWISI*

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Comparatively few studies of the effect of x-ray treatment of a host upon the course of a concomitant infection have been reported. Zinsser and Castaneda (1), by irradiating their animals, successfully increased the susceptibility of rats to typhus rickettsiae, and Liu, Snyder and Enders (2) irradiated mice with similar results. Kolmer, Rule and Werner (3) found that x-ray exposure, in the dosages used, did not render rabbits, guinea pigs, rats, nor ferrets susceptible to the virus of poliomyelitis. Corper (4) showed that thorium x had no effect on experimental guinea pig tuberculosis; Corper and Chovey (5) explain this finding on the basis that antibodies and circulating leucocytes are less important in a chronic infection like tuberculosis than in acute infections produced by pneumococci and hemolytic streptococci, which they showed to be enhanced by thorium x treatment of mice.

In the field of antibody production, Benjamin and Sluka (6) found that in animals irradiated before injection of beef serum the precipitin titer was lower and the antigen disappeared more slowly than in normal animals, but that irradiation four days after the injection of the antigen did not have these effects. L  wen (7) and von Heinrich (8) also found that irradiation does not affect antibody already formed and Hektoen (9) showed that production of lysin for red blood corpuscles was markedly reduced in animals which received large doses of x-ray at the start of immunization, although x-ray treatment given when antibody production was already well established had little or no effect upon the amount of hemolysin formed. He concluded that lymphoid tissue, which is the most susceptible to x-irradiation, is the source of antibodies.

Since, therefore, there is relatively little evidence on the influence of x-rays upon resistance, and that evidence conflicting, it was thought desirable to plan a series of experiments designed to study the effect of x-irradiation of a host upon the course of his infection and upon various aspects of his resistance. *Trypanosoma lewisi* was chosen as the infecting organism for this work because the course of infection with it in its natural mammalian host, the rat, so readily lends itself to quantitative determination. It was hoped that, if x-irradiation were found to influence the resistance of the host, some information might be gained both as to the reason for this effect and

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the normal defense mechanism of the rat to this parasite. While *Trypanosoma lewisi* is not a parasite of man, it is possible that knowledge gained from investigations into host reactions to it may also apply in human trypanosomiasis.

#### MATERIALS AND METHODS

1. *The strain of rats.* All the rats used were of the Sherman strain and were maintained by the Department of Animal Care on a diet devised by Dr. C. A. Slanetz, consisting of:

Ground whole wheat ..	4050 g	CaCO <sub>3</sub> .....	90 g
Commercial casein ...	900	Dried meat scraps (com-	
Skim milk powder ...	600	mercial) .....	600
		Salt butter .....	450 (recently,
			butter 225 g,
			mazola 225 g)

All rats used in the experiments to be described were progeny of animals which had been rendered free of *Bartonella muris* infection by intramuscular injection with neosalvarsan, 0.015 g per 100 g of rat.<sup>3</sup> The rats were kept in a separate room. Their cages, food and bedding were sterilized before use. The cages were cleaned twice weekly and, in addition, fresh sterile cages were supplied once weekly. All rats were individually dusted at intervals of 3 to 4 weeks with "Pulvex," a commercial rotenone-containing powder, and the cage racks stood in cans holding kerosene. At the conclusion of each experiment, one animal of each series was splenectomized and held for three weeks. During this time, spreads of the tail blood were made at frequent intervals and carefully examined, after staining with Giemsa's stain, for evidence of *Bartonella muris*. At no time were any infected erythrocytes found.

2. *The strain of trypanosome.* The strain of *Trypanosoma lewisi* used in this work was kindly supplied by Mr. Henderson of the National Institute of Health. It was one which grew well at room temperature on slants of 10% rabbit blood-nutrient agar, overlaid with nutrient broth. Following intraperitoneal inoculation into normal rats, the prepatent period was 1 to 4 days and the peak of the infection was generally attained on the seventh to the tenth day. Trypanosomes used for infection were obtained either from a seed rat whose blood was diluted with nutrient broth, or from a young culture of *Trypanosoma lewisi*. The infecting doses ranged from 10,000 to 150,000 organisms, all the animals of each group receiving the same dose, as recorded in the protocols below.

3. *Procedure for irradiation.* X-ray exposures were very kindly performed by the late Dr. H. H. Kasabach and his assistants in the Department of Radiotherapy, Presbyterian Hospital. The rats were so placed in cardboard or wooden boxes that they could not climb over each other. The aperture of the target was large enough to cover the surface of the box, so that each rat was irradiated over its entire body. The following factors were used: 200 kv, 25 ma, a filter of 1 mm Al and inherent

<sup>3</sup> Because many of the rats maintained at this institution are infected with the common rat parasite, *Bartonella muris*, a preliminary study was made to determine whether the presence of this infection affected the resistance of rats to x-irradiation. Following irradiation, there was an immediate sharp decrease in circulating leucocytes and a delayed drop in erythrocytes, in all experimental and control rats. For the *Bartonella*-free rats, the average minimum white count was 552 per cu mm, reached in 2 to 4 days, and the minimum red count, 3.1 per cu mm, at 12 to 14 days; for the *Bartonella*-infected rats, the corresponding values were 370 and 2.4. For this reason and because survival time following x-ray tended to be less among the *Bartonella* carriers, it was decided to use only *Bartonella*-free rats for the work to be described.

filter of 0.15 mm Cu; 50 cm target-skin distance. The intensity of radiation was 125 to 140 r per minute, measured in air.<sup>4</sup> The dosage used in each experiment is stated below.

4. *Trypanosome counts.* (a) *Total counts.* Trypanosome counts on the tail blood of infected animals were carried out with a hemocytometer, using Hayem's solution as the diluent, or when the infection was slight, by counting the number of parasites seen in 50 high-power microscope fields ( $\times 450$ ) of whole blood.

(b) *Percentage of dividing parasites.* Two hundred unselected parasites were counted on Giemsa- or Wright-stained blood films. Only those organisms in which a nucleus or kinetoplast was observed to be undergoing division were considered as dividing. A parasite which was apparently reproducing to form more than two new individuals was counted as one dividing organism.

5. *Preparation of vaccine.* Sixteen-day-old rats were intraperitoneally inoculated with 0.1 ml of a 3-day culture of *Trypanosoma lewisi*. Eight days later, when trypanosome counts ranged from 720,000 to 1,400,000 per cu mm of tail blood, the rats were bled from the carotid artery. The blood was thoroughly mixed with sodium citrate solution, diluted with physiological saline and slowly centrifuged to remove all the erythrocytes. Formalin was added to the supernatant trypanosome suspension to make a final concentration of 0.5% formalin (0.5 ml of 40% formaldehyde per 100 ml). For some of the work, part of this suspension was then diluted in 0.5% formalinized saline to give 4.3 million trypanosomes per ml. Since the presence of the formalin apparently rendered injections of this suspension very painful, the remainder of the suspension was centrifuged at high speed, and the organisms were washed and resuspended in saline containing 0.3% crude cresol. There were 4 million trypanosomes per ml in this phenolized vaccine. The sterility of the final suspensions was proved by their failure to cause infection in rats inoculated with them.

6. *Agglutinin titrations.* Rat sera were serially diluted in 0.9% saline solution. 0.15 ml of the vaccine described above (containing 4,000,000 trypanosomes per ml) was added to an equal amount of each serum dilution. After thorough mixing of antigen and serum, the tubes were placed in an incubator (37° C) for one hour and then in a refrigerator (10° C) for 20 to 24 hours. The tubes were examined in the light of a 40-watt bulb and the degree of agglutination estimated. Control tubes of serum 1:10 as well as of antigen suspension 1:2 were carried through the same incubation procedures.

#### EXPERIMENTAL WORK

##### PART I

##### *Effect of X-ray upon Experimental Infections with Trypanosoma lewisi*

Younger rats are known to suffer longer and more intense *Trypanosoma lewisi* infections than older rats (11, 12). For this reason and since the effect of irradiation

<sup>4</sup> In a preliminary experiment, it had been found that larger rats survived higher exposures for a longer time than smaller ones. Since larger animals get proportionally more irradiation because of more scatter and greater absorption, it was apparent that the older rats were capable of sustaining much larger amounts of x-ray than younger ones, and that absolute x-ray dosage could not be used in this work since the magnitude of the effect of irradiation would depend on the age of the recipient. It was found experimentally that the maximum x-ray dosage which uniformly permitted the survival in reasonably good nutrition of normal, uninfected rats was 300, 400, 500 and 500 r, respectively, for 15-, 25-, 35- and 60-day-old rats. It may be mentioned that these values, at least for the oldest group, are somewhat higher than those given by Leach and Sugiura (10) who reported that all 7 of their 2½- to 3½-month-old rats that were exposed to 500 r died within 18 days, some on the 2nd day.



TABLE 1.—Effect of X-irradiation upon the Number of Circulating Trypanosomes

No. of rats	Series	Age when infected (days)	X-ray (r)	No. of trypanosomes in infecting dose	Average parasite counts* on tail blood on designated days after infection																		
					3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24	26	28	
3	D	15	300	150	0	+	++		545	683	1200	1650d	875	597	562	339	373	353	400	303	277	356s	
3	E	15	300	10	+	+			1533	1533	1267	718	...	653	917	463	650	597	508	448	352	393s	
3	D	25	400	150	++	196			159	520	...	1030	...	935+	1150	1050	750	750	650	585	670	600s	
3	E	25	400	10	143				1433	1315	...	893	...	950	690	600	815	533	625	605	320	445s	
3	D	35	500	150	+	73			67	577	...	902	1047	1050+		680+	650	500	...	360	375	480s	
3	E	35	500	10	...	30			...	870	...	425	...	665	595	783	680	455	283s	...	...	...	
3	D	60	500	150	...	345			138	812	...	1092	...	758	718	...	519	513	490	364	377	302	350s
3	E	60	500	10	...	...			...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	D	15	...	150	0	+	++		165	283	293	...	215	212	245	128	109	116	172	82	79	132s	
3	E	15	...	10	+	+			75	468	560	...	...	367	...	408	241	117	47	109	51	54s	
3	D	25	...	150	+	11			33	15	...	13	...	13	32	25	22	15	11	18	13	19s	
3	E	25	...	10	37				233	376	...	195	...	122	126	150	90	78	81	77	70	35s	
3	D	35	...	150	+	15			26	...	...	15	...	12	11	12	21	23	11	16	19	4s	
3	E	35	...	10	+	...			...	69	...	130	64	91	...	43	...	38	118	70	59	45s	
3	D	60	...	150	+	26			104	77	...	40	...	56	43	29	5	+	0s	...	...	...	
3	E	60	...	10	...	117			...	...	173	...	157	109	...	70	102	94	89	101	64	45s	

In each series, all the rats of the same age were litter-mates; Series D was started in July, 1942, E, in Sept.-Oct., 1942.

\* 0 represents absence of trypanosomes in 50 microscope fields ( $\times 450$ ).

++ less than one trypanosome per microscope field ( $\times 450$ ).

1 to 10 trypanosomes per microscope field ( $\times 450$ ).

The figures, 150, 10, 165, etc., represent thousands of trypanosomes/cu mm.

One rat of group died.

Two rats of group died.

|| Infection cleared in 1 rat.

s indicates that rats were sacrificed; d indicates that rats died.

upon the course of the infection might manifest itself variously in animals of different ages, rats were selected from 4 age groups: nurslings (15 days), young rats just being weaned (25 days), adolescents (35 days), and adults (60 days).

*Effect of x-irradiation upon the intensity of infection.* The course of *Trypano-*

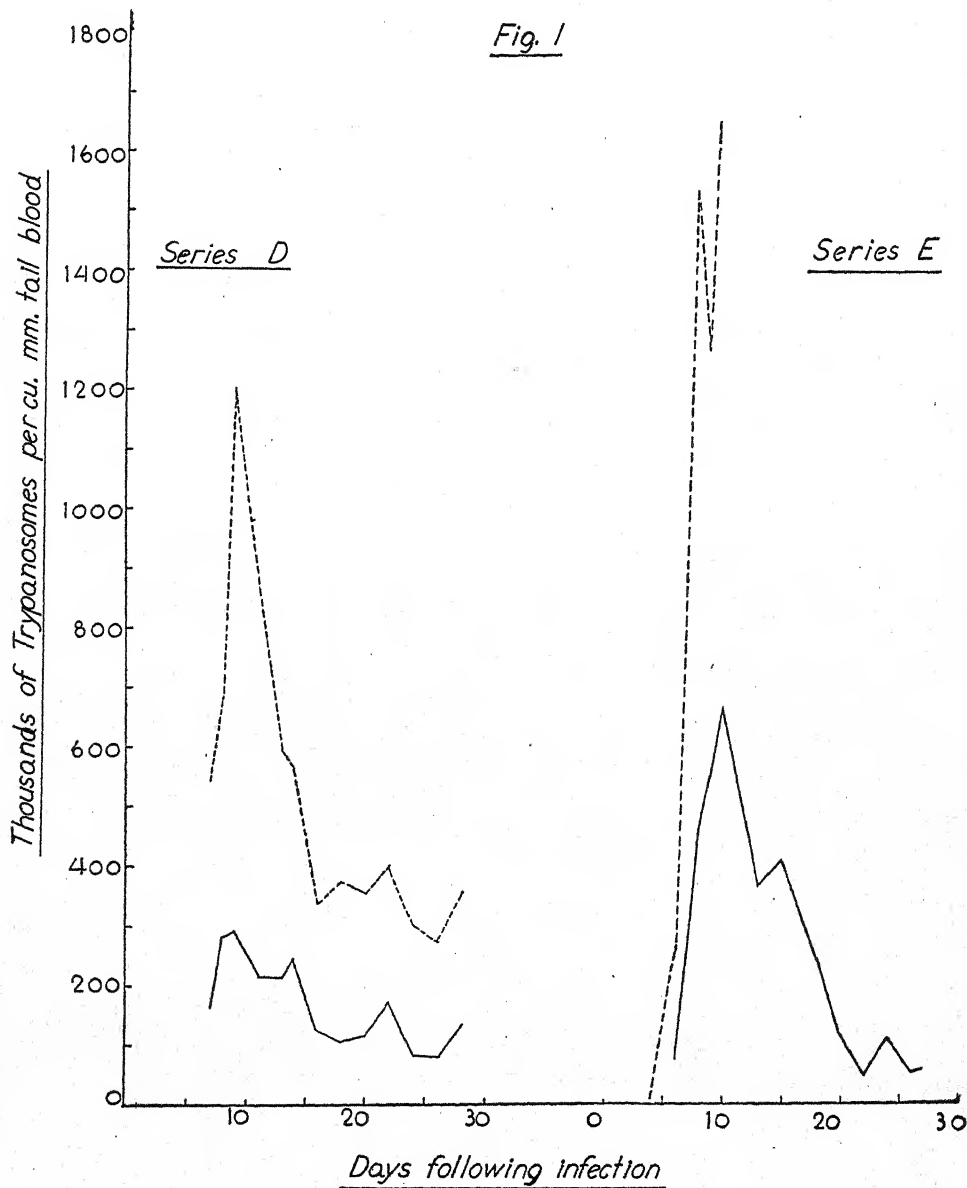


FIG. 1. 15-day-old rats, — infected only; ---- irradiated and infected.

*soma lewisi* infection in 46 rats, ranging in age from 15 to 60 days at the time of irradiation was followed. All the rats were infected within one hour of exposure of the test animals. The effect of the irradiation upon resistance, as revealed by the subsequent trypanosome counts, may be seen in Table 1 and Figs. 1-4.

The prepatent period varied from 1 to 4 days and appeared to be independent both of the size of the infecting dose and the factor of irradiation. The peak of the

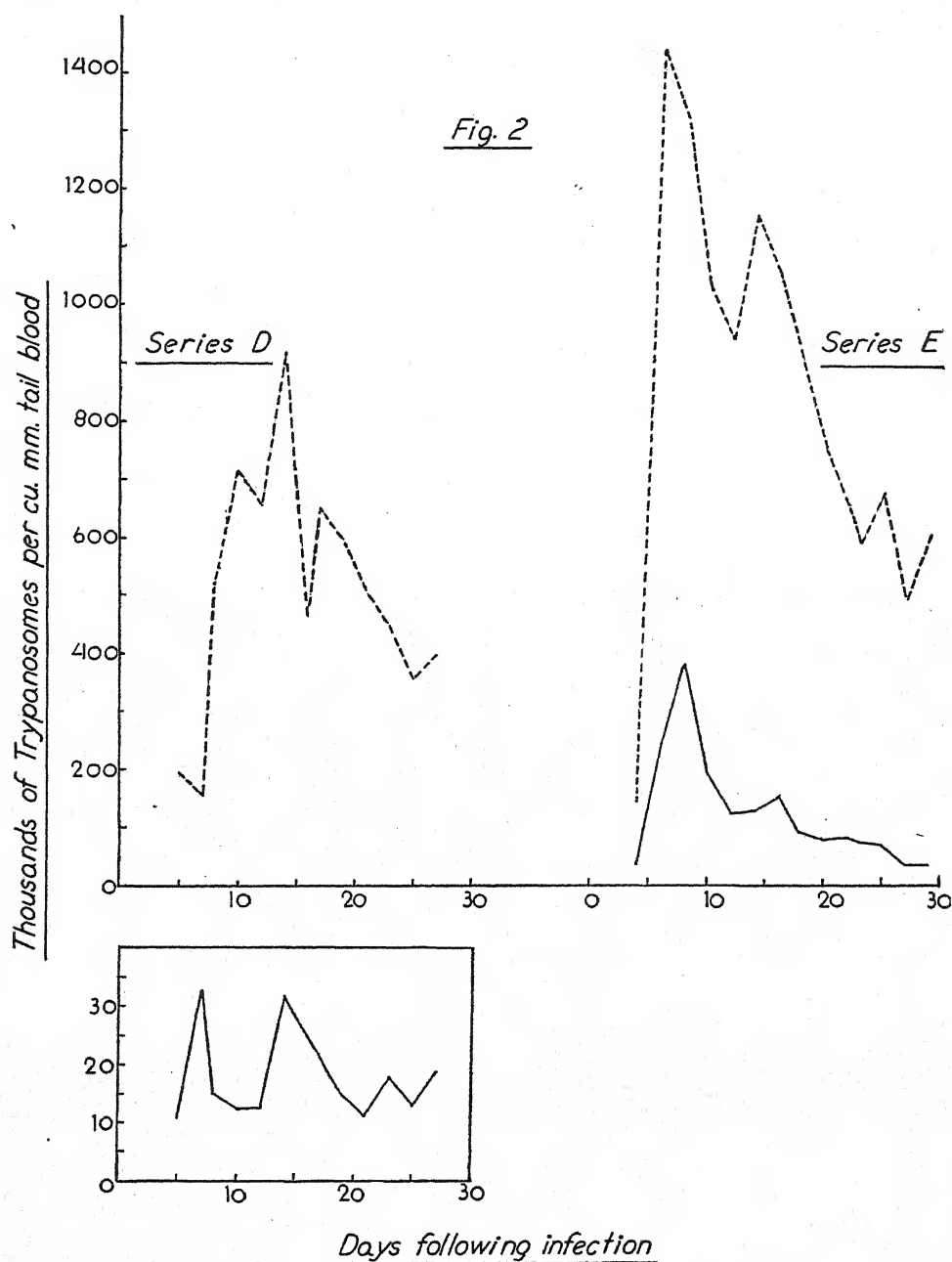


FIG. 2. 25-day-old rats, — infected only; ---- irradiated and infected.

infection was significantly higher in each of the irradiated groups than in their non-irradiated controls. Similarly, the decline in the number of circulating trypanosomes was slower in the irradiated groups. Because the strain of *Trypanosoma lewisi* used

tends to produce a prolonged chronic infection, the experiments were arbitrarily terminated at the end of 27 to 29 days, if the infection had not already disappeared by that time. Although it is therefore not possible to determine whether there was any difference in the duration of infection in the two groups, it is perhaps suggestive that the 3 rats whose infections did clear within 4 weeks were all unirradiated.<sup>5</sup>

*Rat weight.* This index of metabolism was compared in 3 groups of rats, irradiated, infected and both irradiated and infected.

Retardation of growth occurred in the irradiated animals. In each age group, those rats which were infected as well as irradiated made poorer progress than, and never quite caught up to, their irradiated, non-infected littermates. This observation indicates that whereas infection of otherwise normal rats has little if any influence upon the rate of development (15), infection in irradiated rats produces a definite inhibition of growth beyond that imposed by the radiation itself; i.e., irradiation appears to render the rats less capable of normal metabolic processes in the presence of *Trypanosoma lewisi* infection, as well as making them less resistant to the infection itself.

*Mortality.* Of the 23 irradiated infected rats, 8 died of *Trypanosoma lewisi* infection. The mortality rate in these animals was therefore approximately 35%, whereas among the control rats, none of which died, it was nil.

## PART II

### *Factors Contributing to the Irradiation Effect on Trypanosoma lewisi Infection*

Both cellular and humoral factors may play important roles in resistance. In the following experiments an attempt was made to determine which of these factors was so affected by x-ray treatment of the rats that increased susceptibility to *Trypanosoma lewisi* resulted.

*Effect of x-irradiation upon the leucocyte level in Trypanosoma lewisi-infected rats.* The established relationships between blood leucocyte levels and course of infection suggested the possibility that the increased susceptibility of irradiated rats to *Trypanosoma lewisi* might be correlated with the marked drop in circulating leucocytes which follows irradiation, although Culbertson and Kessler (16) who studied the relationship between leucocyte count and agglutinin production in response to *Trypanosoma lewisi* vaccine, found no evidence that an altered leucocyte picture was responsible either for resistance acquired through vaccination or for the much greater resistance exhibited by older rats after vaccination, in contrast with that artificially acquired by younger rats. Duca (11) found that the leucocyte count in *Trypanosoma*

<sup>5</sup> In view of such experiments as those of Patel (13) who showed that 6000 r is the lowest dose which affects the virulence of *Trypanosoma brucei*, and of Halberstaedter (14) whose experiments demonstrated that 12,000 r is required to inhibit the reproduction and therefore the infectivity of *Trypanosoma gambiense*, it appeared unlikely that trypanosomes injected into irradiated rats would be affected by the radiation energy remaining in the host tissues. Nevertheless it was thought advisable to test this possibility. Accordingly, 3 previously infected 35-day-old rats were irradiated when they showed an average of 12,000 parasites per cu mm of tail blood. By the 8th day following exposure, all had trypanosome counts of over 1 million per cu mm; one died on the 13th day and 2 on the 15th day after irradiation. In 3 litter-mates irradiated and infected simultaneously, the circulating parasites reached approximately the same number at the peak of the infection and 2 died, on the 13th and 15th days. Apparently, therefore, there is no significant irradiation effect on circulating parasites when their rat hosts are x-rayed.



*levisi*-infected rats rose and fell parallel with the rise and fall of the number of circulating trypanosomes and the leucocyte response may probably justifiably be taken as an indication of the general cellular response to the infection.

In the irradiated infected rats used in these experiments, the white blood counts

Fig. 3

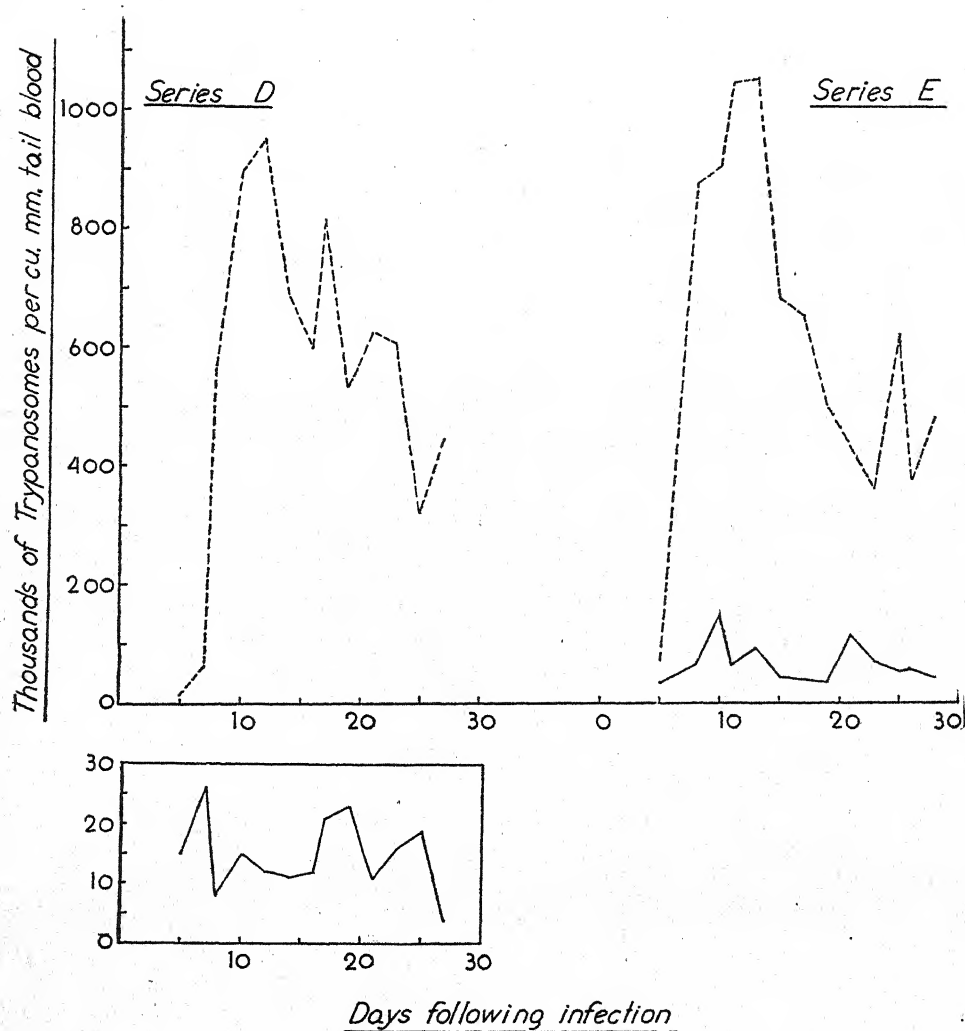


FIG. 3. 35-day-old rats, — infected only; ---- irradiated and infected.

reached a minimum, generally on the 4th or 5th day following irradiation, ranging from about 250 to 1500 leucocytes per cu mm. Shortly thereafter the counts started to rise. On the other hand, the unirradiated infected rats maintained counts of 15,000 to 25,000 per cu mm for the greater part of the acute infection. Table 2 shows

the time relationships between peak of infection, peak of white count and rise in white cells of irradiated animals.

The fact that the rise in circulating leucocytes in the irradiated rats started simultaneously with or before the peak of the infection or the peak white count occurred even in the normal rats probably indicates that the depressing effect of the x-rays upon resistance was not attributable to their influence upon the leucopoietic organs. In the rat, according to the observations of Latta and Ehlers (17), exposure to x-ray sufficient to depress the circulating leucocytes to 1000 per cu mm results in a reversal of the normal lymphocyte-granulocyte ratio from approximately 70% lymphocytes and 20% polymorphonuclear leucocytes to 20% and 70% respectively. In the present experiments, because of the extremely low white counts, running as low as 250 cells per cu mm, coupled with erythrocyte counts ranging to 8 or 9 million per cu mm during a good part of the infection, it was impossible to find sufficient leucocytes on blood smears to give accurate differential results. However, the indication was that the cells of the lymphocytic series were most severely affected by the irradiation. The

TABLE 2.—Effect of x-irradiation upon the leucocyte count in *Trypanosoma lewisi*-infected rats

No. of rats	Age when infected (days)	X-ray (r)*	Average number of days after infection		
			Rise in WBC began	Peak of WBC occurred	Peak of infection occurred
6	15	300	6.5	8.0	9.0
6	15	300	6.5	9.0	9.0
6	25	300	6.5	12.0	10.5
6	25	400	9.0	14.0	14.0
6	35	500	6.5	9.0	7.0
6	35	500	6.5	17.0	12.0
5	60	500	6.5	10.5	6.0
5	60	500	6.5	14.5	14.0

\* X-ray exposure given one hour before infection.

rise in leucocytes which followed the initial drop was probably due to the regenerative powers of the spleen, lymph nodes and bone marrow, which were first demonstrated by Heinecke (18). A precipitous rise and fall in the leucocyte count of an occasional irradiated rat, long after the peak of the infection, was observed during the course of the present work. This may indicate that in some cases there was temporary over-compensation during the regeneration of the leucopoietic centers.

It has been shown above that the circulating leucocytes were probably not responsible for the effect of x-ray in diminishing resistance to *Trypanosoma lewisi*. Intense activity of reticular and endothelial cells in *Trypanosoma lewisi* infections has been demonstrated (19, 20) and it has frequently been shown (21, 22, 23, 24) that macrophage tissue is concerned in the formation of lysin and of ablastin antibody as it is known to be in the formation of other antibodies (25). Those very cells which appear to be responsible, in large measure at least, for resistance to *Trypanosoma lewisi* are also severely affected by irradiation (18, 26, 27). Their importance in resistance is probably not due solely to their phagocytic ability, as originally suggested by Laveran and Mesnil (28). It is still a moot question whether the prime factor is their ability to form a trypanolysin (29, 30), or ablastin in addition to a lysin (20, 31), or whether both factors are involved (22). In an attempt to explain the effect of irradiation on the course of *Trypanosoma lewisi* infection upon a humoral basis, several questions suggested themselves. Was the effect due to an inability (or

delayed ability) to produce antibody, or was it due to an inability to utilize antibody that was present?

*Effect of x-irradiation of rats upon ablastin production.* Taliaferro (20, 23, 32) and Coventry (31) have stated that in "non-pathogenic" trypanosome infections, the

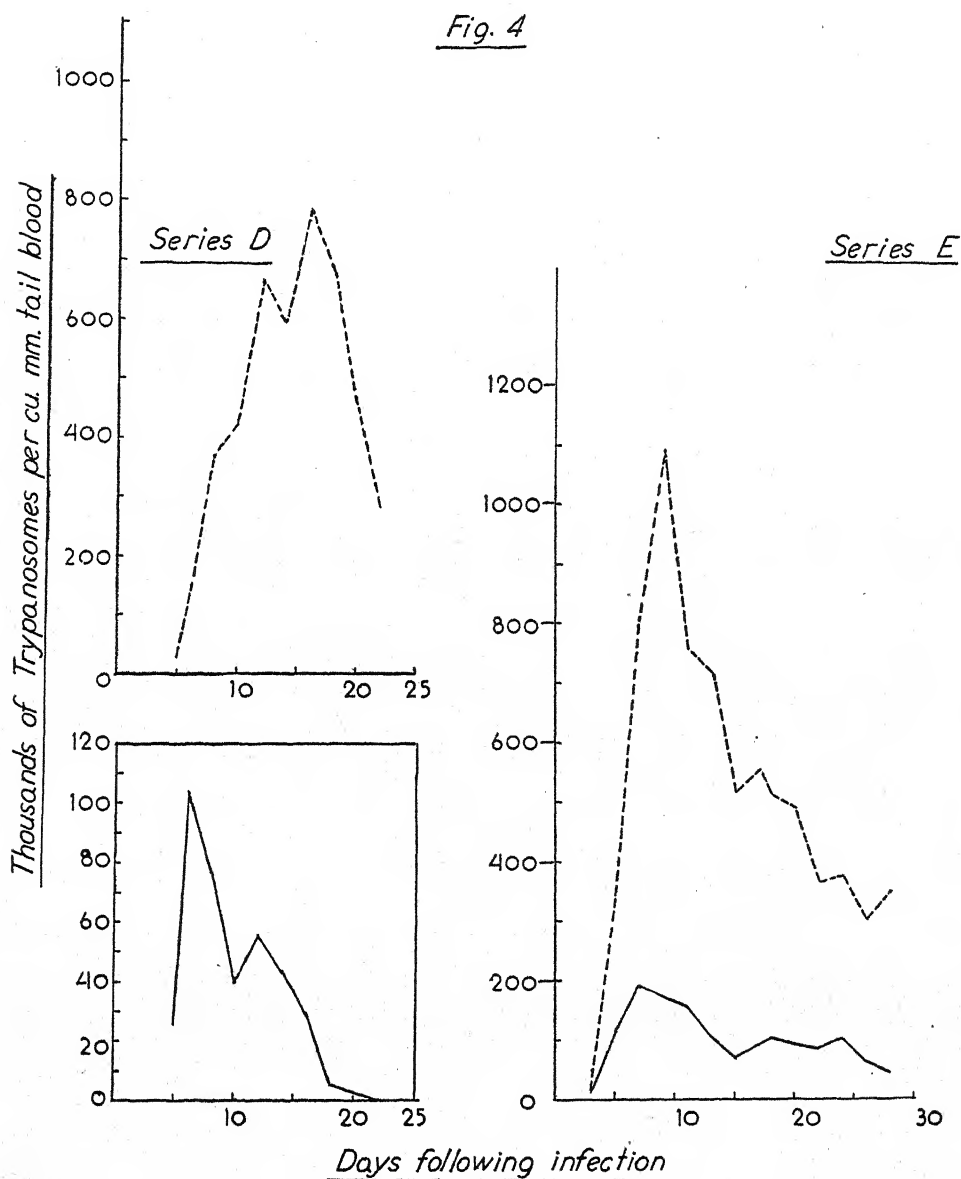


FIG. 4. 60-day-old rats, — infected only; ---- irradiated and infected.

initial resisting mechanism is the production by the host of an ablastin antibody which completely inhibits reproduction. This is followed by the periodic production of a trypanolysin, so that the infection clears by a succession of number crises. Taliaferro

TABLE 3.—Effect of x-irradiation of rats upon ablastin production

Rat No.	Age when infected (days)	X-ray (r)	Total parasite counts per cu mm of tail blood and the percentage of dividing parasites on designated days following infection*†																	
			4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28
E4 ...	15	300	...	...	192 8.0	...	1600 8.5	1100 9.5	1200 10.0	d.	...	...	...	...	...	...	...	...	...	...
E5 ...	15	300	...	...	169 5.5	...	1400 5.0	1800 4.0	2100 6.0	d.	...	...	...	...	...	...	...	...	...	...
E6 ...	15	300	...	...	400 13.0	...	1600 5.5	900 8.0	d.	...	...	...	...	...	...	...	...	...	...	...
E25 ..	25	400	68 10.5	...	1450 7.0	...	1600 7.5	...	810 6.0	...	860 6.0	d.	...	...	...	...	...	...	...	...
E27 ..	25	400	260 9.0	...	1450 4.0	...	945 5.0	...	1180 5.0	...	886 3.5	d.	...	...	...	...	...	...	...	...
E13 ..	35	500	...	50 6.0	...	...	620 3.5	...	1300 3.0	940 1.0	...	400 8.0	d.	...	...	...	...	...	...	...
E15 ..	35	500	...	120 5.5	...	...	1200 6.5	...	640 8.0	1100 8.0	d.	...	...	...	...	...	...	...	...	...
D14 ..	15	300	...	...	...	...	590 3.0	1200 2.0	...	400 3.5	...	475 1.0	...	360 0.0	446 0.0	300 0.5	460 0.0	380 0.5	236 0.0	200 0.0
D9 ...	25	400	...	44 2.5	...	...	59 4.0	460 5.0	830 3.5	...	445 0.5	...	800 1.0	600 1.0	800 1.0	545 0.5	525 0.5	560 0.0	400 0.5	390 1.5
D2 ...	35	500	...	...	...	...	68 6.5	525 3.0	1050 3.0	...	700 1.5	...	680 1.5	760 4.5	900 3.0	440 1.5	610 1.5	380 1.0	140 0.0	310 0.5
D19 ..	60	500	...	...	36 3.0	...	460 2.0	...	340 2.0	...	630 2.0	...	650 2.0	710 0.0	710 0.5	540 0.5	215 1.5	s.	...	...
D16 ..	15	None	...	...	...	230 3.0	300 2.0	450 4.0	...	315 3.0	...	250 2.0	232 3.5	125 0.0	120 0.0	180 0.0	265 1.0	92 0.5	96 0.5	96 0.5
D11 ..	25	None	...	28 6.0	...	30 6.0	18 5.0	...	18 1.5	...	18 1.5	...	32 4.5	26 1.0	20 1.5	23 1.5	7 1.5	8 ...	10 ...	28 0.5
D5 ...	35	None	...	20 5.0	...	30 2.5	40 2.5	...	22 2.0	...	8 0.0	...	14 5.0	20 5.0	30 3.5	30 4.0	8 ...	25 ...	28 1.5	8 ...
D22 ..	60	None	...	...	96 5.0	...	80 1.5	...	240 3.0	...	233 2.0	...	25 1.0	15 1.0	8 ...	5 ...	Clear	...	...	...

\* Upper figure in each row = parasite counts in thousands per cu mm of tail blood.

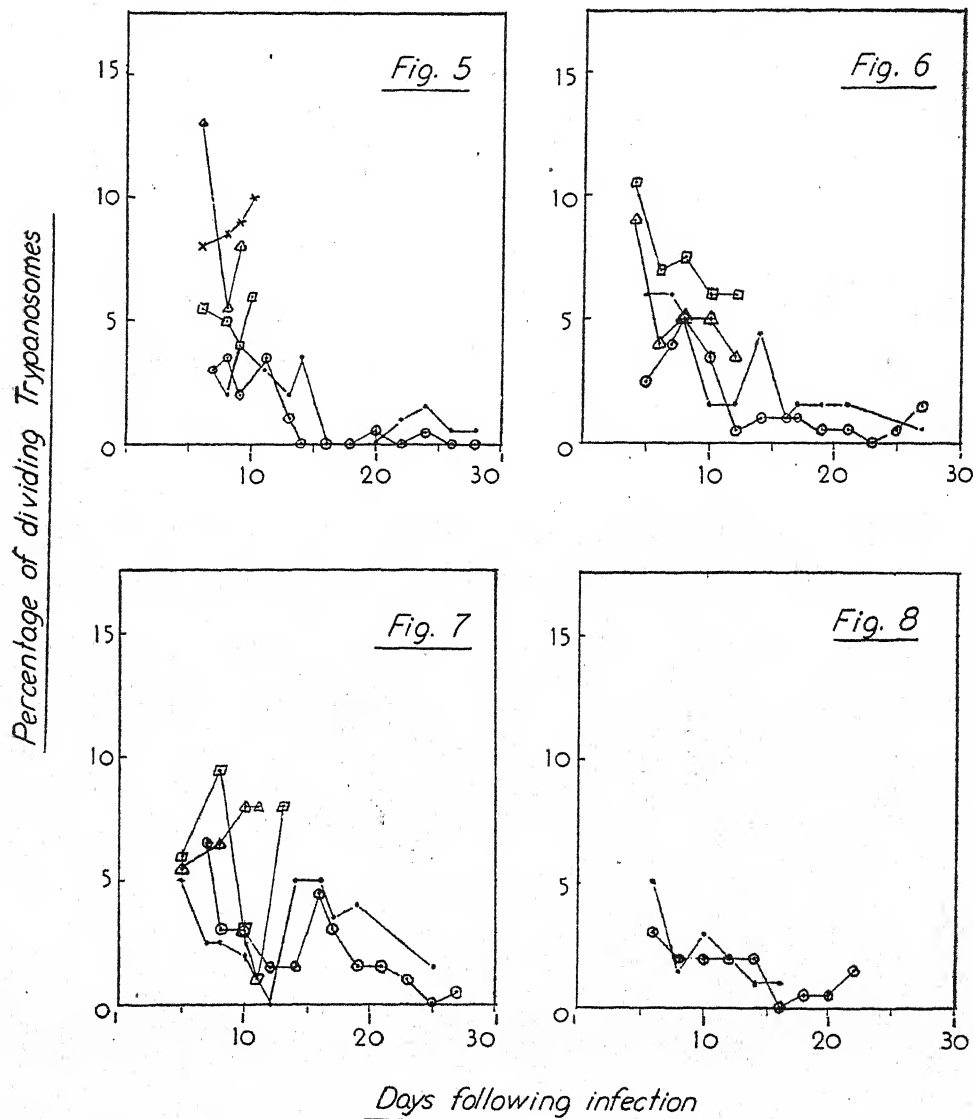
† Lower figure in each row = percentages of dividing forms in 200 parasites examined in stained blood films.

d. indicates that rat died.

s. indicates that rat was sacrificed.



(23) cites cases of apparently lethal infections with *Trypanosoma lewisi* in which reproduction of the parasites was never completely inhibited, and Culbertson and Wotton (33) showed that among a group of infected rats only those died which exhibited a high proportion of dividing parasites. The amount of ablastin present



FIGS. 5-8. Rate of reproduction of trypanosomes. FIG. 5. 15-day-old rats. FIG. 6. 25-day-old rats. FIG. 7. 35-day-old rats. FIG. 8. 60-day-old rats.

- rats infected only.
- rats irradiated and infected, which survived.
- x—x } rats irradiated and infected, which died.
- }
- △—△ }

can be estimated by determining the proportion of parasites undergoing division. It is of interest to do this since a comparison of successive total parasite counts does not necessarily give an accurate picture of the course of the infection, for a gradual increase in the number of organisms may mean either that the parasites are reproducing slowly, or that a rapid rate of reproduction is almost paralleled by the rate of destruction. The greater intensity of infection noted in irradiated rats therefore reflects either their relative inability to inhibit reproduction, or a decreased ability to destroy the parasites, or both. Table 3 and Figs. 5 to 8 show the rate of reproduction of the trypanosomes infecting representative rats of each age group.

Among the rats which survived the infection, there seemed to be no significant difference between experimental and control animals in the percentage of dividing forms occurring at different stages of the infection. The fact that those rats which died (all young) permitted a high level of reproduction may mean that in them, the irradiation was effective enough to destroy even the ablastin, which younger rats normally produce in smaller amounts, whereas in those which survived, ablastin was successfully produced although lysin production was delayed or diminished. It is difficult to determine whether this indicates that the ablastin and lysin are different manifestations of the same antibody, the manufacture of the ablastic property being less injured by irradiation than that of the lytic, or whether there are two antibodies, produced by different cell types which in turn showed a differential sensitivity to x-ray. It was clear, however, that irradiation exerted a marked effect upon the lytic antibody (since a high level of infection persisted longer in the irradiated animals, even though the reproduction rate was no higher than in the controls) as well as upon the ablastic property of those rats which died.

*Effect of x-irradiation upon antibody production in response to Trypanosoma lewisi vaccine.* Of the 19 rats used in this experiment, 9 were irradiated and vaccinated and 7 were vaccinated but not irradiated. Three other rats served as additional controls, one being irradiated and the other two neither irradiated nor vaccinated. Six vaccine injections (4.0 or 4.3 million trypanosomes per 100 g rat) were given at 3-day intervals, the first coming on the day of irradiation. All the rats were infected with 130,000 to 150,000 trypanosomes on the 5th day following the last vaccine inoculation. Some of these animals were bled for serum samples just prior to the first vaccination and on the day of infection; all were bled at the end of the infection or at the conclusion of the experiment.

Of the 9 irradiated and vaccinated rats, 7 were successfully infected, whereas the parasite did not become established in any of the non-irradiated animals after immunization. It will be noted in Table 4 that the irradiated rats fell into two groups on the basis of their resistance following vaccination, one group exhibiting marked infection, the other showing either slight or no infection. Despite this difference in ability to develop immunity in response to vaccine, however, it was apparent that irradiation had the effect, in most instances, of sharply diminishing the ability to produce protective antibody. This finding was reinforced by the agglutinin titers of serums obtained five days after the last vaccine injection, shown in Table 5. In the control rats this was 1:320, while in the irradiated rats it was only 1:40, although two of the latter were nevertheless resistant to infection.

*Effect of x-irradiation upon passive protection against Trypanosoma lewisi.* These experiments were carried out to ascertain whether irradiation influenced the

TABLE 4.—Effect of  $\alpha$ -irradiation upon antibody production in response to *Trypanosoma lewisi* vaccine

Rat No.	Age when vaccine series began (days)	X-ray (r)	Vaccine*	Parasite count† on tail blood on designated days following infection‡													
				2	4	6	8	10	12	14	16	18	20	22	24	26	28
F1	31	400	+	0	0	++	290	363	d.	800	580	486	418	320	390	370	284
F5	26	400	+	0	0	++	640	980	925	112	100	114	86	112	128	62	98
F7	26	400	+	0	0	++	230	211	179	32	52	35	40	36	22	26	24
J2	35	500	+	+	+	75	80	48	32	76	58	60	60	36	72	77	61
J4	35	500	+	+	+	88	153	90	68	32	14	11	10	28	8	35	6
F6	26	400	+	0	0	+	36	42	31	+	0	0	0	0	0	0	0
F8	26	400	+	0	0	+	4	25	21	+	0	0	0	0	0	0	0
J1	35	500	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0
J3	35	500	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F3	31	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F4	31	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F9	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F10	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F11	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J5	35	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J6	35	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J7	35	...	.	0	+	49	36	31	18	10	0	8	40	36	27	10	0
J8	35	...	.	0	0	+	23	80	78	44	52	11	40	100	21	10	0
J10	35	500	.	+	35	132	412	230	154	83	92	72	108	100	21	0	0

\* All the vaccinated rats received 6 intraperitoneal injections of 4.0 or 4.3 million trypanosomes per 100 g of rat, at 3-day intervals.

† Key to parasite counts: Same as Table 1.

‡ Infecting dose of 130,000 or 150,000 trypanosomes given 5 days after last vaccine injection.

TABLE 5.—Effect of x-irradiation upon agglutinin titer following vaccination

Rat No.	Age when vaccine series began (days)	X-ray (r)	No. of vaccine injections at 3-day intervals	Infecting dose given 5 days after last vaccination (thousands of trypanosomes)	Became infected	Highest dilution of serum ( $\times 2$ after addition of antigen) in which agglutination occurred		
						Before vaccination	5 days after last vaccination	33 days after last vaccination
F5	26	400	6	150	Yes	....	.....	1:40
F6	26	400	6	150	Yes	....	.....	1:40
F7	26	400	6	150	Yes	....	.....	1:40
F8	26	400	6	150	Yes	....	.....	1:80
J2	35	500	6	150	Yes	1:40	1:40	1:160
J4	35	500	6	150	Yes	1:80	1:40	1:160
J1	35	500	6	150	No	1:20	1:40	1:40
J3	35	500	6	150	No	1:20	1:40	1:40
F4	31	...	6	150	No			1:320
J5	35	...	6	150	No	1:40	1:320	1:80
J6	35	...	6	150	No	1:20	1:320	1:80

efficacy of immune serum administered for protective purposes. Culbertson (34) showed that 0.25 ml of *Trypanosoma lewisi* antiserum per 10 g rat weight, given intraperitoneally on the day of infection, completely protected rats of various ages. Twelve of the 20 rats used for this experiment received intraperitoneal injections of pooled immune rat serum. Of these 12 rats, 8 had been irradiated within the previous hour. All, plus the controls, received the infecting dose of 100,000 trypanosomes subcutaneously to eliminate the possibility of immediate union of antibody and organisms in the peritoneal cavity.

Series	Age (days)	Rat no.	X-ray (r)	Serum no.	Days following infection:				Trypanosomes at height of infection, thousands per cu. mm.
					0	20	40	60	
G	25	1	400	57					
		2	400	57					
		3	400	57					
		4	400	57					
		5	-	57					
		6	-	57					
		7	400	-		died			950
		8	400	-		died			2090
		9	-	-					320
		10	-	-					440
H	15	1	300	70					
		2	300	70					
		3	300	70					138
		4	300	70					
		5	-	70					140
		6	-	70					139
		7	300	-					1300
		8	300	-					1540
		9	-	-					1850
		10	-	-					1080

FIG. 9. Effect of x-irradiation upon passive protection against *Trypanosoma lewisi*. Length of rectangle indicates period of observation; blacked-in areas indicate duration of *Trypanosoma lewisi* infection. Serum 57: pooled from serum of 3 rats 25 days old at infection and 3 rats, 60 days, all nearly recovered at bleeding 4 weeks later; dose, 0.25 ml per 10 g rat. Serum 70: pooled from 3 rats 25 days old at infection, 2, 15 days and 2, 60 days, all either nearly or just recovered at bleeding 4 weeks later; dose 0.30 ml per 10 g rat to allow for previous dilution with merthiolate.



It was apparent (Fig. 9) that the 15-day-old rats of series H were less well protected by the serum given them than were the 25-day-old rats of series G. Whereas all of the latter were completely protected, 3 of the former became frankly infected, although only one of these had been irradiated. Even in these animals, however, the serum clearly had some effect, since in each case the prepatent period was prolonged, the duration of the infection markedly reduced, and the peak of the infection very much lower than in their litter-mate controls. Since even those rats which showed infection were partially protected, it may be assumed that both sera contained ablasic as well as lytic antibody. Taliaferro (35) has shown that serum containing ablasic is as effective in splenectomized blockaded rats as in normals. However, in normal animals, the passive ablasic immunity which lasts only a few days is replaced by a developing, active ablasic immunity whereas in the reticulo-endothelium-depleted animals, active ablasic immunity does not develop. This might explain the present finding that infection, where it did develop, was considerably delayed; that is, the infection developed after the disappearance of the passively introduced ablasic. Apparently most of these rats were able to manufacture ablasic and later lysis. Although some were not, this ability was not affected by the x-ray treatment.

Two factors may have been operative in the difference in protection afforded the two groups (G, H) of rats, namely the actual protective titer of the serum used and the age of the rats. Since the sources of the sera were approximately equivalent, there should not have been much difference between their protective values. To explain the observed differences in protective action on the basis of age differences between the two groups necessitates reconciling the discrepancy between the observations of Culbertson (34) that rats as young as 10, 15, and 20 days of age were completely protected by immune serum, and the present results. The assumption that his sera were more potent than those used here would resolve the difficulty, for it is well known that in immature animals, the macrophage tissue, of fundamental importance in the utilization of antibodies introduced into the body (35), is not fully developed functionally (36). Possibly the cooperation offered by immature macrophage tissue may not be sufficient, in the presence of a relatively weak antiserum, to resist infection completely.

For the purposes of this study, the important conclusion to be drawn from the data given in Fig. 9 is that irradiation did not significantly influence the effectiveness of antibodies introduced into the body simultaneously with the infecting dose. This may indicate, if true, that the effect of irradiation on the macrophage tissue is not exerted all at once but that before the effect is fully developed, the reticulo-endothelial system has already made its necessary contribution to the protective action of the serum.

*Effect of x-irradiation upon latent Trypanosoma lewisi infection.* Permanent resistance to reinfection by some protozoa (e.g., malarial plasmodia, *Endamoeba histolytica*, *Trypanosoma brucei*) has been shown to be of a latent infection (premunition) type and this may be true also in other cases in which proof of this type of immunity has not been obtained. Perla and Marmorston (36) state that since splenectomy does not stimulate recrudescence of the infection in a recovered rat, *Trypanosoma lewisi* infection is apparently not an example of a true latent infection. However, the essential mechanism of resistance to reinfection with *Trypanosoma lewisi* is still under discussion. It has been attributed to phagocytosis of the few

trypanosomes which may migrate into the blood stream from the site of inoculation (28), to ablastin plus phagocytosis (22) and to lysin plus ablastin (29, 30, 31). In recent work, Augustine (37) has shown that trypanosomes (*Trypanosoma lewisi*) introduced into the peritoneal cavity passed with equal ease through the lymph nodes and into the blood stream of normal and immune rats. He further showed (38) that immunity to reinfection with *Trypanosoma lewisi* seems not to be due to the presence of ablastin since, with a massive infecting dose, the trypanosomes which remained in the blood stream for 2 days could be seen to divide. Apparently the failure of the parasites to increase in number was caused by the opsonic or agglutinative effect of the parasitocidal antibody.

It was felt that the x-ray technique used in this work might serve to demonstrate whether latent infection does in fact occur with *Trypanosoma lewisi*. Accordingly, of 7 litter-mate rats, infected when very young, 5 were irradiated as soon as their blood stream infection had cleared. Frequent subsequent blood examinations showed no trypanosomes, except in the case of one irradiated rat. Although examination of this rat revealed a single circulating parasite on the sixth and seventh days following irradiation, it was apparently suffering an atypical infection because weeks later, when no parasites could be seen microscopically, its blood was still able to infect clean rats although the blood of an irradiated litter-mate could not. From these experiments, one can conclude only that if there is a latent stage of *Trypanosoma lewisi* infection, x-ray did not inhibit the defense mechanisms to the point where the organisms were driven into the blood stream.

#### DISCUSSION

The present study has shown that x-irradiation depresses the resistance of rats to *Trypanosoma lewisi* infection. The effect of the rays was to increase the intensity of the infection and to prolong the period of high trypanosome levels in the blood. The striking effect of irradiation upon the leucocytes, especially of the lymphocytic series, was apparently unrelated to its effect upon resistance. The fact that there is marked activity of reticulo-endothelial cells in *Trypanosoma lewisi* infections, coupled with the knowledge that this tissue is severely affected by x-ray, suggested the possibility that the inhibiting effect of irradiation upon resistance might be due to its interference with humoral mechanisms. In pursuing this hypothesis it was found that while there was no significant difference in the protective action of immune serum administered to normal and irradiated rats, the ability of the rats to produce antibody, both ablastic and lytic, was markedly diminished by irradiation. X-ray treatment inhibited antibody production not only in the presence of infection, but also in response to a series of vaccine injections. From this it appeared that the x-rays, known to destroy macrophage tissue, diminished resistance to *Trypanosoma lewisi* by decreasing the amount of antibody produced, since it is fairly well established that the initial defense mechanisms in this infection are humoral in nature. It may be concluded that macrophage tissue, particularly in its antibody-producing capacity, is of paramount importance in the resistance of rats to this infection.

Infection in irradiated animals tended definitely to inhibit their growth, indicating that irradiation not only made the rats more susceptible to infection but also rendered them less capable of normal metabolic processes in the presence of *Trypanosoma lewisi*. There may well be a direct relationship between the deficient metabolism of the animal as a whole and its increased susceptibility to infection.

The experimental results obtained by x-irradiation of host animals suggest that this technique might profitably be employed in studies where a partial and temporary elimination of the reticulo-endothelial system is advantageous. Provided the dosage is not excessive, regeneration of the injured tissue, especially in the bone marrow and lymph nodes, is quite complete (27).

#### SUMMARY AND CONCLUSIONS

X-irradiation of rats increased the severity of their *Trypanosoma lewisi* infection. In 46 rats of four age groups, 15, 25, 35 and 60 days old, respectively, at the start of the experiment, the peak of the infection ranged from 2 to 8 or more times as high in the irradiated animals as in the controls.

The ability of rats to produce ablastic antibody against *Trypanosoma lewisi* was markedly diminished by x-ray treatment. In those irradiated rats which failed to survive the infection, the proportion of reproducing parasites remained at approximately 5 to 8%, whereas in the survivors, both irradiated and control, the proportion declined in about 10 days to 0 to 2% from an initial high of 4 to 6%. Trypanolysin production also seemed to be affected by irradiation as indicated by the greater duration of a high level of infection even in those survivors whose ablastic level was diminishing.

Vaccination with a formalinized trypanosome suspension completely protected 100% of 7 normal rats from a subsequent infecting dose of 130,000 trypanosomes, whereas only 22% of 9 previously irradiated rats were similarly protected.

Previous x-irradiation of rats did not significantly influence the effectiveness of protective antibodies introduced simultaneously with the infecting dose of *Trypanosoma lewisi*.

The sharp reduction in circulating leucocytes which followed x-ray treatment appeared not to be a factor in the decreased resistance of irradiated animals, since the leucocyte level had returned to approximately the normal value at the time of the peak of infection.

Irradiation of rats recovered from *Trypanosoma lewisi* infection did not produce evidence of a latent stage of the infection.

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## STUDIES ON THE VITAMIN REQUIREMENT OF TAPEWORMS<sup>1</sup>

C. J. ADDIS AND ASA C. CHANDLER

The vitamin sources and requirements of tapeworms present an interesting problem not only from the parasitological point of view but also from the standpoint of the physiology of the host. The possible absorption of vitamins from the mucosa of the intestine may account for some of the pathogenic effects of parasitism by tapeworms, and there is an additional point of interest in an apparent relation between the vitamins and the sex hormones, as pointed out by the work of Chandler (1943).

Very little work has been done on the vitamin requirement of the cestodes. Hager (1941) found that there was a marked decrease in the egg production of *Hymenolepis diminuta* in rats when the hosts were kept on a milk diet and when soybean meal was substituted for yeast. A diet deficient in both vitamin B<sub>1</sub> and the G complex caused a very marked decrease in the egg output, with loss of the worms in some cases. There was no significant decrease in the egg output when only B<sub>1</sub> was eliminated from the diet, but the lack of the G complex alone did cause a decrease. Hager suggested that there is a factor in yeast associated with the G complex which is necessary for normal egg production by the rat tapeworm, but this factor is not necessarily a member of the G complex.

Chandler (1943), working with *Hymenolepis diminuta* in rats, studied the effect of variations in the diet of the host on the worm as determined by counting and measuring the worms after autopsy. He found that this worm is totally independent of protein in the diet of the host and drew the conclusion that the worms absorbed the nitrogenous substances directly from the mucous membrane. He suggested that this dependence of the tapeworm on the mucosa of the host for nitrogenous materials might explain the cause of the stunting effect of crowding, which has been repeatedly reported. He also found that the lack of vitamins in the diet, with or without the provision of proteins, had a marked effect on the establishment of the worms in female rats, while in male rats there was no apparent effect in either case. Also, there was a marked stunting in the growth of the worms in female rats on a vitamin-deficient diet with provision of proteins, but in female rats which lacked both vitamins and proteins the worms grew to the normal size. In male rats on a vitamin-deficient diet, with or without the provision of proteins, there was no effect on the growth of the worms.

In further experiments, Chandler showed that this worm is dependent on some factor contained in autoclaved yeast in the diet of the host, but the lack of the fat-soluble vitamins A, D, and E and vitamin B<sub>1</sub> had no effect on the growth of the worms in female rats.

Chandler suggested that there must be some intricate involvement of the female sex hormones with protein metabolism and vitamin utilization, which as yet has not been explained. He also expressed the view that tapeworm toxicity may be due to the absorption of proteins and vitamins, and possibly hormones or other substances, from the mucosa of the intestine of the host by the worms, instead of the absorption of toxic products of the worms by the host.

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The present investigation was carried out as a continuation of the work of Chandler (1943) to determine more exactly the relationship between the vitamin content of the diet of the host and the establishment and growth of the worms. The method used was similar to that of Chandler (1943). The rat tapeworm, *Hymenolepis diminuta*, was used in all the experiments and the hosts were laboratory-bred albino rats. The results were based on the number and size of the worms after autopsy.

The grain beetle, *Tenebrio molitor*, was used as the source of the cysticercoids. The beetles were infected by mixing the eggs obtained from a stock supply of infected rats with shaved apple and feeding it to the beetles. After 19 to 21 days the beetles were teased apart and cysticercoids were removed and washed. Each rat was then given 10 mature cysticercoids by means of a pipette. In the following experiments only female rats were used, since Chandler (1943) showed that there was no effect of a vitamin-deficient diet on the worms in male rats. The rats were between 3 and 4 months old and weighed between 150 and 200 grams at the beginning of the experiments. Autopsies were run on the rats 14 days after being infected since at this time no segments have yet been shed, although the worms have as great an average length as they do if removed from the host at later periods. The worms were washed out of the intestines with warm water and were allowed to relax in cold water for two to three hours, after which they were counted and measured. All worms measured possessed a terminal segment and a scolex.

In the following experiments the rats were placed on diets varying in their vitamin content. The basic portion of the diet for six rats per day was as follows:

Fisher Ruffex <sup>2</sup> .....	10 gm
Casein, vitamin-test .....	18 gm
Dextrose .....	25 gm
Corn starch .....	25 gm
Corn oil .....	10 gm
Salt mixture .....	4 gm

For a complete diet for six rats per day, 9 gm of Harris' or Fleischmann's brewer's yeast were added to supply the B complex, and 3 ml of a mixture of 30 ml of cod-liver oil, 25 ml of wheat-germ oil, and 4 ml of halibut-liver oil to provide the fat-soluble vitamins A, D, and E. For a diet containing no B<sub>1</sub>, 9 gm of autoclaved yeast was used. For a diet containing vitamin B<sub>1</sub> alone, a 1-mgm tablet of thiamin chloride was dissolved in 10 ml of 20 per cent alcohol and added to the basic portion for six rats per day.

The basic portion of the diet was mixed in such quantity as usually to last the length of the experiment. Each day the proper amount of this portion was weighed and the vitamin content added. On days when the entire amount of food given the rats the day before was not eaten, enough of the basic portion was added to bring it back to its normal weight, but the full amount of the vitamins was added to insure adequate amounts of them.

#### EXPERIMENT 1

In the first experiment four groups of six female rats were used. Each rat was weighed, fed 10 cysticercoids, and started on its experimental diet the same day,

<sup>2</sup> The Fisher Ruffex is composed mainly of alpha cellulose and was used to provide bulk; its food value is practically nil and all the vitamins have been destroyed or removed.

allowing no time for the depletion of the host's vitamin store. Group I was given a complete diet; group II was given a diet which lacked all the vitamins; group III was given a vitamin B<sub>1</sub>- and G-complex-deficient diet; and group IV was put on a diet deficient in vitamins A, D, E, and the G complex. After 14 days the rats were autopsied and a comparison of the number and size of the worms was made.

The results of this experiment, as shown in Table 1, show that the lack of vitamins in the diet of the host has some effect on the establishment and growth of the tapeworms even when no time is allowed for the depletion of the host. In group II, where there was a complete lack of all the vitamins, the number of worms that were able to establish themselves was somewhat less than that of the worms in the rats on the other diets. There was also a stunting in the growth of these worms. In group III, rats on a vitamin B<sub>1</sub>- and G-complex-deficient diet, the average number of worms was the same as that in the rats on a complete diet, but the growth of the worms was stunted as much as it was in the vitamin-deficient rats. In group IV,

TABLE 1.—No preliminary depletion

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete) . . . .	+ 2.6	8.2	251.6-358.3	321.4	± 8.13	2,635.5	1.56
II (no vitamins) . . .	- 8.0	4.7	118.3-290.0	231.6	± 20.87	1,088.5	1.11
III (no B <sub>1</sub> or G complex) . . .	- 6.1	8.2	147.5-302.5	231.0	± 15.43	1,894.2	1.08
IV (no A, D, E, or G complex) . .	- 6.1	6.0	183.6-295.0	247.1	± 13.34	1,482.6	1.15

\* Ratios of  $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$ :

1. I to II = 4.03.      2. I to III = 5.17.      3. I to IV = 4.79.

no vitamins A, D, E, or G complex, the number of worms was lower than that in groups I and III but not as low as in group II. The average length of the worms was about the same as that of the worms in the rats with no vitamins and in those lacking vitamin B<sub>1</sub> and the G complex.

Although this experiment showed that the lack of vitamins in the diet of the host increases resistance to the establishment of the worms and causes a stunting in the growth of the worms that become established, the effect on the worms was not as marked as might have been expected. Consequently a second experiment was planned in which the host was put on a depletion diet for a certain length of time.

## EXPERIMENT 2

In this experiment a depletion period of 4 days was allowed before the cysticercoids were fed. The four diets used in experiment 1 were repeated, and four additional groups were put on diets containing the remaining combinations of the three groups of vitamins. Group V was put on a diet containing none of the vitamin G complex; group VI, no vitamin B<sub>1</sub>; group VII, no vitamins A, D, or E; and group VIII, no vitamins A, D, E, or B<sub>1</sub>.

A study of Table 2 will emphasize the conclusions of experiment 1. The number of worms and their size in the vitamin-deficient rats were reduced still further. In the four groups containing none of the G complex, i.e., groups II, III, IV, and V, there was a loss of weight in the rats, as might be expected from such diets, and a



definite stunting in the growth of the worms. It can be seen that in group V, lacking the G complex only, and in group II, lacking all the vitamins, there was produced the greatest stunting in growth, while in groups III and IV, lacking vitamins B<sub>1</sub> and vitamins A, D, and E, respectively, as well as the G complex, the reduction in the length of the worms was not as great. Although the difference between the four groups is not statistically significant, as seen from the ratios, there is a suggestion that the absence of vitamins A, D, E, and B<sub>1</sub> may be favorable to the growth of the worms. The possible effect of these vitamins will be discussed further below. In the three remaining groups, VI, VII, and VIII, containing the G complex, the growth of the worms approximated that of the worms in the rats on a complete diet.

It can be seen that the lack of all the vitamins definitely decreases the number of worms that succeed in establishing themselves, but just what vitamin or group of vitamins is concerned is not very clear. Judging by the results in groups V and VII, it seems to be the fat-soluble vitamins, A, D, and E and the G complex that are involved, but in groups III and VIII, which lacked the G complex and the fat-soluble

TABLE 2.—4 days depletion.

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete) ..	+ 16.5	7.8	220.9-376.8	326.6	± 15.62	2,545.8	1.59
II (no vitamins)	- 19.4	3.5	69.0-198.5	126.0	± 21.43	491.4	0.78
III (no B <sub>1</sub> or G complex) ..	- 11.1	6.2	150.0-239.2	210.0	± 14.78	1,302.0	0.99
IV (no A, D, E, or G complex)	- 13.1	5.0	80.0-215.8	171.9	± 14.97	859.5	0.98
V (no G complex)	- 10.2	5.5	126.3-195.0	140.3	± 9.82	771.7	0.82
VI (no B <sub>1</sub> ) .....	+ 10.5	6.4	195.0-266.3	255.3	± 9.12	1,663.9	1.37
VII (no A, D, or E)	+ 8.9	4.8	258.0-379.8	324.1	± 16.68	1,555.7	1.46
VIII (no A, D, E, or B <sub>1</sub> ) ....	+ 10.9	6.8	308.9-344.8	318.9	± 13.44	2,168.5	1.42

\* Ratios of  $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$ :

- |                     |                      |                       |                         |
|---------------------|----------------------|-----------------------|-------------------------|
| 1. I to II = 8.31.  | 5. I to VI = 3.93.   | 9. II to IV = 1.76.   | 13. IV to V = 1.77.     |
| 2. I to III = 6.29. | 6. I to VII = 0.10.  | 10. II to V = 0.61.   | 14. VI to VII = 3.62.   |
| 3. I to IV = 7.06.  | 7. I to VIII = 0.36. | 11. III to IV = 1.82. | 15. VI to VIII = 3.93.  |
| 4. I to V = 10.11.  | 8. II to III = 3.23. | 12. III to V = 3.94.  | 16. VII to VIII = 0.24. |

vitamins, respectively, and also vitamin B<sub>1</sub>, the number of worms present was close to that of the controls, and in group VI, lacking vitamin B<sub>1</sub> only, there was likewise no significant reduction in the number of worms present. It would seem from these data that the lack of either vitamins A, D, and E or the G complex inhibits the establishment of the worms, while there is a suggestion that the absence of vitamin B<sub>1</sub> is favorable to their establishment.

Because of the questions which arose from this experiment and in order to try to make the picture clearer, another experiment was planned in which the host was depleted of its vitamins for a longer time.

### EXPERIMENT 3

In this experiment the same diets used in experiment 2 were repeated, allowing 17 days for the depletion of the host before feeding the cysticercoids.

A study of Table 3 shows that a longer depletion period in the host intensifies the effects of the lack of certain vitamins in the diet. In the rats on a diet deficient in the G complex there was a marked stunting in the growth of the worms, while in

the three groups which received an adequate amount of the G complex the worms reached a greater length than those in the controls. These results corroborate the need of the vitamin G complex in the diet of the host for the normal growth of the tapeworm, and provide further evidence that the worms grow larger, rather than smaller, in the absence of the fat-soluble vitamins and of vitamin B<sub>1</sub>.

In this experiment, the decrease in the number of worms established in the absence of the fat-soluble vitamins is more marked than in experiments with shorter depletion periods. The absence of the G complex also seems to have some effect on the establishment but is not as pronounced as the lack of the fat soluble vitamins. The absence of the G complex along with that of vitamins A, D, and E intensifies the reduction in the number of worms established. In diet VI, where the rats were on a vitamin-B<sub>1</sub>-deficient diet, there is no significant effect on the number of worms present.

TABLE 3.—17 days depletion

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete)	+17.3	8.0	304.0-356.7	328.3	± 16.48	2,626.4	1.58
II (no vitamins)	-18.6	3.2	56.3-133.0	101.1	± 8.58	323.5	0.69
III (no B <sub>1</sub> or G complex)	-18.2	5.7	107.4-249.0	190.9	± 16.54	1,088.1	0.97
IV (no A, D, E, or G complex)	-15.1	3.0	141.7-197.0	166.4	± 9.58	499.2	0.96
V (no G complex)	-15.5	5.0	82.0-184.6	128.9	± 11.13	944.5	0.68
VI (no B <sub>1</sub> )	+11.1	6.7	328.4-424.9	339.4	± 6.42	2,274.0	1.57
VII (no A, D, or E)	+10.2	3.8	305.0-572.4	388.5	± 6.58	1,476.3	1.61
VIII (no A, D, E, or B <sub>1</sub> )	+ 9.6	5.3	366.5-480.2	399.4	± 5.73	2,116.8	1.62

\* Ratios of  $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$ :

- |                   |                    |                     |                       |
|-------------------|--------------------|---------------------|-----------------------|
| 1. I to II=12.21. | 5. I to VI=0.63.   | 9. II to IV=5.10.   | 13. IV to V=2.55.     |
| 2. I to III=5.90. | 6. I to VII=3.38.  | 10. II to V=1.95.   | 14. VI to VII=5.34.   |
| 3. I to IV=8.48.  | 7. I to VIII=4.09. | 11. III to IV=1.28. | 15. VI to VIII=6.98.  |
| 4. I to V=10.02.  | 8. II to III=4.83. | 12. III to V=3.12.  | 16. VII to VIII=1.25. |

## DISCUSSION

The experiments described above indicate clearly that the rat tapeworm, *Hymenolepis diminuta*, is dependent upon the G complex in the diet of its host for normal growth, as previously suggested by the work of Hager (1941) and Chandler (1943); the stunting effect on the growth of the worms becoming progressively more marked with an increase in the length of the depletion period prior to the infection of the host.

It is probable that tapeworms require the same vitamins required by other organisms; they may acquire them: (1) from the unassimilated food of the host, (2) by absorption from the mucous membranes of the host itself, or (3) by synthesis. The first source has definitely been eliminated by the work of Chandler (1943) and by the present investigation for all except the G complex. Absorption from the mucous membranes, or synthesis, seems to afford an adequate source for all except the G complex or some part of it, since diets deficient in all but the G complex have no harmful effect on the growth of the worms. Since the effect of the absence of the G complex in the food of the host became progressively more marked as the host's depletion period was increased prior to infection, it is a logical

deduction that the missing vitamin or vitamins are not synthesized by the worms but are obtained by absorption from the host's mucous membrane. Only in rats well supplied with the G complex in the daily food is an adequate amount available, but even after long depletion enough can be absorbed by the worms to allow them to grow at a slower rate. It seems logical from this that an infection of tapeworms in a host living on a diet deficient in this group of vitamins would intensify the avitaminosis. Experiments are in progress now to determine the component or components of the G complex that are necessary for the normal growth of the worms.

Since the lack of the fat-soluble vitamins and B<sub>1</sub> in the diet of the host had no stunting effect on the growth of the worms after a long depletion period, and even produced an increase in the size of the worms over that of the controls, the possibility suggests itself that the worms may be able to synthesize any of these vitamins which they need for normal maintenance of growth and health. However, yeast autoclaved for an hour is not entirely depleted of thiamin, and "vitamin-test" casein contains traces of it. In a personal communication Dr. R. J. Williams informs us that samples of such casein tested in his laboratory contained from 0.13 to 0.28 micrograms of thiamin per gram, about one-tenth to one-twentieth of that found in a good food mixture. The worms could, therefore, obtain a small amount of thiamin from this source. Also, as pointed out by Dr. Williams, the tissues of an animal fed on a thiamin-deficient diet do not immediately fall to a low level of thiamin content, so the worms could possibly have derived enough of this vitamin from the mucous membrane of the host even after several weeks of depletion.

The increase in size of the worms in rats on diets deficient in the fat-soluble vitamins is in accordance with observations made by Ackert, McIlvaine, and Crawford (1931) on the nematode, *Ascaridia lineata*, in chickens on a vitamin-A-deficient diet. They attributed this increase in size to the partial paralysis of the intestine, thus producing a richer intestinal flora in the vitamin-A-deficient chickens than in the controls. In the present investigation it was noted at autopsy that the intestines of the rats deficient in the fat-soluble vitamins or vitamin B<sub>1</sub> were more compact and contained more fecal material than did the intestines of the other rats, indicating weakened peristalsis. This observation is in accordance with that of Ackert, McIlvaine, and Crawford (1931) and the several reports that vitamin B<sub>1</sub> is corrective of certain types of constipation. It seems probable that the increase in the growth of the worms in these cases, if not entirely accounted for by the smaller number of worms present, might be due to the weakened peristalsis. This partial paralysis of the intestine would allow the worms to become established in a more anterior position which, by analogy with the result obtained by Burlingame and Chandler (1941), would be more favorable for growth. No data were obtained on the position of the worms in the rats, so this conclusion is only tentative. Weakened peristalsis and possible establishment of the worms in a more favorable anterior position might also explain the moderate increase in size of the worms in the rats on a diet deficient in vitamins A, D, E, and the G complex (groups IV) and on a diet deficient in vitamin B<sub>1</sub> and the G complex (groups III) as compared with the worms in the rats on a diet deficient in the G complex alone (groups V). A study of the size of the worms in the individual rats does not warrant the conclusion that the increase in size is due to a smaller number of worms present. There is little correlation between the number of worms and the average length of the worms in the individual

rats of a dietary group. For example in groups III and IV in experiment 2 the number of worms and average lengths are as follows:

Group III			Group IV		
No. of rat	No. of worms	Average Length	No. of rat	No. of worms	Average Length
1	9	117.4	1	7	157.0
2	7	239.2	2	7	137.0
3	6	218.3	3	6	215.8
4	6	150.0	4	4	171.0
5	1	85.0	5	3	150.0
6	0	0.0	6	3	80.0

Apparently the number of worms present, up to 9 or 10 worms, has little influence on the length to which the worms grow during the first 14 days. The increase in length with diminution in numbers reported by Chandler (1939) is apparently due mainly to growth after the first 14 days.

The establishment of tapeworms would seem to depend upon the proper evagination of the scolices and their attachment upon the mucous membrane of the wall of the intestine. Exactly what causes the evagination in the host is not known, but it is probably dependent upon the digestive juices or the pH of the gut, and any disturbances along this line would affect the establishment of the worms.

The experiments above show that the lack of vitamins A, D, and E decreases the number of worms established. This diminution in the number of worms present in rats on a diet deficient in the fat-soluble vitamins is in contrast to the results of Ackert, McIlvaine, and Crawford (1931), who found that the resistance of chickens to the intestinal nematode, *Ascaridia lineata*, was lowered by a vitamin-A-deficient diet. They attributed this decrease in resistance to the partial paralysis of the intestine, thus giving the worm a longer time in which to attach itself. Chandler (1943) got no significant difference in the number of worms that became established in rats on a diet deficient in vitamins A, D, and E from that of his controls and stated that the worm is independent of the fat-soluble vitamins in the diet of the host. The rats used by Chandler were depleted for only two days before being infected, which might account for his results.

In the case of cestodes, the partial paralysis of the gut would apparently have no effect on the establishment of the worms if evagination of the scolices had not occurred. From work done on the evagination of tapeworm cysts *in vitro* by the use of bile salts (Edgar, 1941), it would seem that the proper secretion of bile in the host would be necessary for the normal evagination of the cysts in the intestine. It is possible that a vitamin-A deficiency interferes with a normal secretion of bile. If it did, the evagination of the scolices might not occur except near the entrance of the bile duct into the intestine, where the concentration of the bile would be the highest, other cysticercoids passing on through the intestine without evaginating. Such cysticercoids as did evaginate would then be established in a favorable anterior position as postulated above.

The smaller number of worms present in the rats on a G-complex-deficient diet may be due to their being eliminated after initial establishment, either because of a tendency to shift position in an unfavorable environment, or to a weakening of the musculature.



## SUMMARY

1. To test the effects of the presence or absence of various vitamins and vitamin combinations on the establishment and growth of *Hymenolepis diminuta* in rats, a series of three experiments was made, with no depletion period, 4 days depletion, and 17 days depletion, respectively, prior to infection. Groups of six female albino rats were infected with 10 cysticercoids each and autopsies made on the fourteenth day.

2. The average number of worms per rat was reduced by a diet deficient in either the fat-soluble vitamins or the G complex and was reduced still further when both of these vitamins were missing. It is suggested that the effect of the lack of the fat-soluble vitamins may be due to some disturbance in the secretion of bile, thus preventing the proper evagination of the cysts. The absence of vitamin B<sub>1</sub> has very little, if any, effect upon the establishment of the worms.

3. The experiments corroborate previous evidence that the lack of some factor associated with the G complex in the diet of the host causes a marked stunting in the growth of the worms. This becomes more marked the longer the depletion period of the host prior to infection, suggesting that the necessary component is absorbed from the host's mucous membrane, and is not obtained in adequate amount in a depleted host. The lack of vitamins A, D, and E or B<sub>1</sub> in the diet of the host causes an increase in size of the worms present. It is suggested that this increase in size over that of the controls is the result of the partial paralysis of the intestine, brought about by the vitamin deficiency, thus enabling the worms to become established in a more favorable anterior position. Since the worms grow in a perfectly normal manner in the absence of any vitamin in the diet of the host except something associated with the G complex, even after a long depletion period which should reduce them in the tissues with which the worms are in contact, the possibility of synthesis of these vitamins by the worm is suggested.

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# THE FLEA GENUS *THRASSIS* AND SYLVATIC PLAGUE, WITH THE DESCRIPTION OF *T. BRENNANI* N. SP.<sup>1</sup>

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During the past decade entomologists and bacteriologists of far Western United States have been conducting extensive investigations on the fleas of native rodents and the incidence of sylvatic plague among these animals. Of the reports published, the résumé by Eskey and Haas (1939) gives the most comprehensive analysis of this relationship. In this report, it is indicated that, of the fleas under observation, species of the genus *Thrassis* far exceeded any others. Likewise, incident to sylvatic plague studies, Jellison (1937) found specimens of *Thrassis petiolatus* (Baker) and *Thrassis pandorae* Jellison in much greater abundance than other fleas collected. It is more than a probability that members of the genus *Thrassis* can be closely associated with the occurrence, or possible occurrence, of plague among native rodents.

According to literature, sylvatic plague has so far been observed only in the far Western areas of the United States, but it is of interest to note that Eskey and Haas (l.c.) have suggested the possibility of its incidence east of the Rocky Mountains. Whether it will be recorded may depend in part on the flea fauna of rodents in other areas that are under observation. Recently Hubbard (1943) described the first known *Thrassis* (*T. princei*) east of the Rockies. This flea was taken from the Great Plains grasshopper mouse, but as Dr. Hubbard suggested, its true host is probably the 13-lined ground squirrel of that area. This record, and the one reported on in this paper, indicates that the genus *Thrassis* may in future sylvatic plague observations and studies be as important in other areas as it has been proven to be in the far West. Following is a list of the known species of the genus and their usual hosts. To date, there are sixteen (16) valid species and three (3) questionable subspecies, all from the Western Nearctic:

- Thrassis acamantis* (Roths.), ex marmots.
- Thrassis arizonensis arizonensis* (Baker), ex antelope ground squirrels.
- Thrassis arizonensis littoris* (Jordan), ex round-tailed ground squirrels.
- Thrassis bacchi* (Roths.), ex 13-lined ground squirrels.
- Thrassis desertorum* Stewart, ex antelope ground squirrels.
- Thrassis fatus* (Jordan), ex 13-lined ground squirrels.
- Thrassis francisi* (C. Fox), ex Yakima ground squirrels.
- Thrassis gladiolis gladiolis* (Jordan), ex round-tailed ground squirrels.
- Thrassis gladiolis caducus* (Jordan), ex antelope ground squirrels.
- Thrassis howelli howelli* (Jordan), ex marmots.
- Thrassis howelli utahensis* Wagner, ex marmots.
- Thrassis jellisoni* Hubbard, ex Oregon ground squirrels.
- Thrassis pandorae* Jellison, ex Montana ground squirrels.
- Thrassis pansus* (Jordan), ex ground squirrels and skunk.
- Thrassis petiolatus* (Baker), ex red digger ground squirrels.
- Thrassis princei* Hubbard, ex grasshopper mouse (?).
- Thrassis rockwoodi* Hubbard, ex Oregon ground squirrels.
- Thrassis spenceri* Wagner, ex marmots.
- Thrassis stanfordi* Wagner, ex marmots.

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<sup>1</sup> Contribution from the Eighth Service Command Laboratory, Fort Sam Houston, Texas.

<sup>2</sup> 1st Lieutenant, Sanitary Corps, Army United States, Entomologist.

Although Eskey and Haas (1.c.) have demonstrated transmission of native rodent plague experimentally with some species of *Thrassis*, much needs to be done for the remainder. Some points of interest in regard to *Thrassis* and sylvatic plague

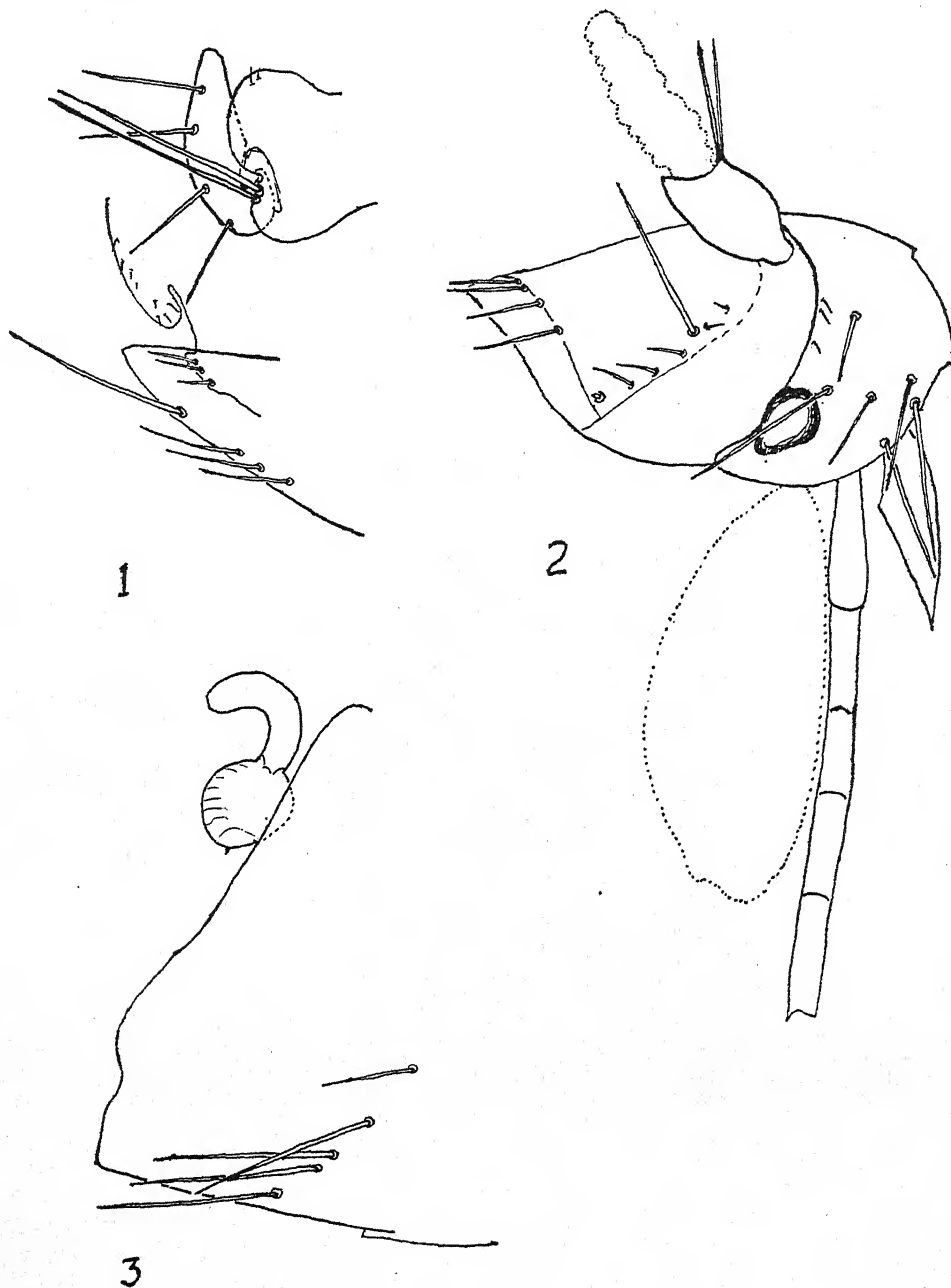


PLATE I

- FIG. 1. *Thrassis brennani* Aug., finger, clasper, sternites IX and VIII of holotype male.  
 FIG. 2. *Thrassis brennani* Aug., head, holotype male.  
 FIG. 3. *Thrassis brennani* Aug., spermatheca, sternite VII of allotype female.

transmission are the host specificity and life habits of the genus. Occasionally, they will be found on other small mammals associated with the host, as pointed out by Hubbard (1.c.), but cannot be collected consistently from such associates. Yet they should be regarded as ideal vectors because they are present in such great numbers and can apparently withstand periods off the host and be found in or around its burrow. In a colony of ground squirrels, for instance, it would not be unusual to expect that each individual *Thrassis* may in its lifetime have an opportunity of feeding on a number of members of the colony. The fact that these fleas can be found at burrow entrances has been taken advantage of by many collectors. By rolling a strip of cotton on the end of a stick and poking it into burrows, a number of fleas can be captured. The writer has made use of this method often but has not been able to obtain species of *Thrassis* as readily as specimens of *Hoplopyllus anomalus* (Baker) and *Diamanus montanus* (Baker). It may also be of interest to note here that the writer has never been attacked by any *Thrassis*, nor are there any certain records that other workers have, but he has frequently been so by *Hoplopyllus anomalus* (Baker), and has recorded (1943) one positive record of *Diamanus montanus* (Baker) attacking man. It can be assumed, however, that species of *Thrassis* are of importance in the transmission and dissemination of sylvatic plague among their native hosts.

With the above information in mind, the following description of a new *Thrassis* is of added interest. This record definitely establishes the genus *Thrassis* east of the Rockies, and also indicates it can be readily taken from the native rodents of this area. During the past year, Dr. J. M. Brennan, while conducting field activities, incident to Bullis fever work, for the Post Surgeon, Fort Sam Houston, Texas, in conjunction with the U. S. Public Health Service, has had the opportunity of collecting ectoparasites from various mammals and birds of this area of the state. From the 13-lined ground squirrel, taken on the grounds of Fort Sam Houston, Dr. Brennan obtained the following flea, which is described and named in his honor.

Family DOLICHOPSYLLIDAE

*Thrassis brennani* n. sp.

Holotype male

*Head:* Frontal notch high, acuminate; preantennal region with two (2) rows of bristles, the upper row with three (3) bristles, the one on genal margin much larger than other two (2), the lower row with three (3) bristles, the center one half the size of the two (2) outer; eye elliptical, not heavily pigmented; maxillae sharply acuminate; labial palpi five (5) segmented, longer than fore-coxa; postantennal region with two (2) large bristles on margin of antennal groove (the posterior bristle broken in type), five (5) smaller bristles also along margin.

*Thoracic and abdominal segments:* Pronotal ctenidium of eighteen (18) slender spines; tergal teeth present as in other members of the genus; a single, large antepygial bristle; tergite VIII reduced, cone-shaped; process of clasper rounded, not reaching apex of finger, with few small setae on apical margin; finger with inner margin broadly angulate, apex narrow, posterior margin evenly rounded with four (4) large bristles; ventral lobe of sternite IX short, shallow, without modified bristles, three (3) normal bristles in a row at apex; sternite VIII sharply pointed, with one (1) large bristle well down from apex, followed by three (3) smaller bristles; penis short, broad near middle, spring without one-half of a full turn.

*Legs:* Tarsus V with five (5) distinct lateral plantar bristles; coxa III rather broad, short; chaetotaxy as in other members of the genus.

Allotype female

*Head:* Frontal notch not as distinct as in male; preantennal region with two (2) rows of bristles, the upper row with two (2), sub-equal, one near antennal groove, one on genal margin, lower row with three (3) bristles, the middle smaller than the two (2) outer; remainder of head as in male.



*Thoracic and abdominal segments:* Pronotal ctenidium as in male; tergal teeth present, as in other members of the genus; two (2) large, equal antepygidial bristles; style short, not twice as long as greatest width; sternite X angulate with many large bristles; sternite VII variable, with indication of a shallow sinus (that in type injured), the upper lobe smaller than the lower, with five (5) bristles in a row, the upper one smaller than other four (4); spermatheca globular, the arm not swollen distally.

*Legs:* Tarsi and rest of legs as in male.

*Holotype:* A male, collected by Dr. J. M. Brennan, Fort Sam Houston, Bexar County, Texas, 8 April 1943, from *Citellus tridecimlineatus texensis*. Deposited in the U. S. National Museum, Washington, D. C.

*Allotype:* A female, collected and deposited as above.

*Paratypes:* Five (5) males, thirteen (13) females collected as above, retained in the Eighth Service Command Laboratory, Fort Sam Houston, Texas.

*Type host:* *Citellus tridecimlineatus texensis*.

*Type locality:* Fort Sam Houston, Bexar County, Texas.

*Remarks:* This new species is close to *Thrassis princei* Hubbard, from which it can be distinguished on the character of the modified segments in the males only, the females of *T. princei* are unknown. Host determination is that of Captain W. J. Hamilton, Jr., Sanitary Corps, formerly at Headquarters, Eighth Service Command.

The writer wishes to express appreciation to his Commanding Officer, Colonel Harvey R. Livesay, M.C., for his constant interest and encouragement in the preparation of this paper.

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CITTOTAENIA SANDGROUNDI, A NEW ANOPLOCEPHALID  
CESTODE FROM A JAVANESE TREE DUCK\*

HELEN EDITH DAVIS

The tapeworms of this study are one of several lots collected in the Dutch East Indies by Dr. J. H. Sandground (formerly Curator of Helminthology, Museum of Comparative Zoology, Harvard College) and kindly presented to Dr. Hughes. The label with the worms indicates that they were taken from *Dendrocygna javanica* (Horsfield) in Batavia in October 1938 and were preserved in seventy per cent alcohol.

The specimens, consisting of two fragmentary strobilae, only one of which has a scolex, were stained in borax carmine and mounted *in toto* in Clarite.

*Cittotaenia sandgroundi* n. sp.

(Figs. 1-11)

*Description:* The strobila with scolex has 332 proglottids, none of which are gravid, and is 65 mm long; the other has 288 segments, some of which are partially gravid, and is 71 mm long. Maximum width 3.72 mm. Margins moderately serrate. Body rather thick—no portion of either worm being sufficiently thin for satisfactory study under oil-immersion objective. Scolex (severely flattened under cover-glass pressure) 120  $\mu$  long to rear margins of suckers and 243  $\mu$  wide. An unarmed rostellum occurs; it comprises recognizable cortical and medullary regions and is 117  $\mu$  long and 63  $\mu$  wide. Suckers 81 (78-87)  $\times$  71 (67-75)  $\mu$ . Neck about 394  $\mu$  long (measured from rear margins of suckers to first distinct line of segmentation) and 330  $\mu$  in minimum width.

Proglottids clearly demarcated, all many times wider than long; the youngest 28  $\times$  398  $\mu$ ; mature ones trapezoidal, 338 (306-360)  $\mu$  long, 2.80 (2.70-2.86) mm wide anteriorly, and 2.96 (2.84-3.04) mm wide posteriorly; gravid ones 380 (370-400)  $\mu$  long, 3.23 (3.20-3.42) mm wide anteriorly, and 3.47 (3.35-3.64) mm wide posteriorly. Two pairs of longitudinal excretory canals, situated about one-fourth width of strobila from respective lateral margins—ventral canals very broad, slightly undulant, and vaguely discernible; dorsal ones slender, more serpentine, clearly defined, and situated almost entirely in regions directly dorsal to the ipsilateral ventral canals; no transverse commissures observed.

Genital apertures paired, marginal, far anteriad, and not salient. Genital atria somewhat cup-shaped, largest near their medial sides; 71 (64-78)  $\mu$  long laterally, 84 (78-90)  $\mu$  long medially, and 65 (45-75)  $\mu$  deep in proglottids 179 to 184 from scolex.

Protandrous. Reproductive systems completely paired except for a common uterus. General loci of gonads roughly trisect width of segments. Genital ducts dorsal to excretory canals. Lateral portions of vagina and cirrus pouch in extreme anterior portion of segment. Postero-peripheral margin of each proglottid so overlaps anterior margin of the next as to obscure the vagina and cirrus pouch of the latter. Female genital pore ventral to male pore and almost impossible to identify except in segments having the margins turned up so as to show the cirrus pouch and vagina in optical cross section.

Unless otherwise stated the measurements listed below for reproductive organs were taken from ten mature proglottids.

Ovary, coarsely and irregularly lobed; anteroventral to testes; 111 (90-135)  $\mu$  in maximum diameter. Vitellarium also irregularly and coarsely lobed, immediately behind ovary, and about half as large as that organ in average diameter. Mehlis' gland immediately dorsal to ovary. Common uterus in mature segments very narrow, thin walled, and comprised of a rather straight transverse canal located somewhat anterior to middle of segment and traversing almost its entire width dorsal to excretory vessels and ovaries, and a pair of short stems leading directly posteriad

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to oötypes; in our oldest segments (only partially gravid) the transverse canal has become unevenly enlarged, ranges from 9 to 82.5  $\mu$  in diameter, and contains eggs (10.5–13.5  $\mu$  in diameter) in scattering groups of varying numbers. Leading inward from the atrium the vagina apparently follows the posterolateral surface of the distal two-thirds of the cirrus pouch; thence it passes forward by a gentle S-curve under the pouch to the anterior surface of the segment; now, as a more clearly visible narrow canal, it crosses over the excretory vessels; next, describing a gentle arc, it reaches the oötype. A portion of the vagina between the excretory tubes and the ovary is somewhat dilated as a seminal receptacle 115 (97–135)  $\times$  31 (28–48)  $\mu$ .

Testes, spheroidal; usually four, rarely as few as two but never more than four observed; variously (often somewhat transversely) grouped dorsoposterosublaterad to ovary; 60 (45–75)  $\mu$  in maximum diameter. Vas deferens, formed by the union of short vasa efferentia that arise on anterodorsal surfaces of testes, passes forward over or near the ovary and follows course of vagina anterolaterad to region dorsal to excretory canals where it expands into a small spheroidal external seminal vesicle [50 (43–57)  $\mu$  in diameter in 5 examples] which is connected with the cirrus pouch by a short, tightly folded S-curve. Cirrus pouch, very prominent, elongate, subcylindrical, nearly straight in mature segments, and heavily muscular; with cirrus only slightly protruded, 575 (447–600)  $\times$  105 (99–112)  $\mu$ ; extends directly mediad almost to, rarely a little beyond, poral excretory vessels. There are apparently two internal seminal vesicles arranged in series in the medial end of the pouch, the proximal one [60 (57–75)  $\times$  32 (27–45)  $\mu$  in seven specimens] being the smaller and more clearly defined. Cirrus stout and very prominent; subcylindrical and tapering gradually from broad base to truncate tip; densely covered with fine spines which vary in form from comparatively stout hooklets 5–6  $\mu$  long over most of the organ to hair-like setae up to 18  $\mu$  long about its base; 167 (156–180)  $\mu$  long, 58 (46–71)  $\mu$  wide at base, and 24 (21–27)  $\mu$  wide at tip, where fully protruded in subgravid segments; never seen to be fully retracted even in very young segments. The fully exerted cirrus, observed only in our oldest segments, is seated in a thick-rimmed collar-like receptacle which seems to represent a portion of the floor of the atrium which has been partially everted and rolled outward about its base and set off by a constriction.

*Host:* *Dendrocygna javanica* (Horsfield).

*Locality:* Batavia, Java, Dutch East Indies.

*Cotype specimens:* Several microscope slides bearing whole mounts of one scolex and fragments of two strobilae in U. S. Nat. Mus. Helm. Coll., No. 36899.

The genus *Cittotaenia* Riehm, 1881, contains tapeworms parasitic as adults in birds and mammals. Baer (1927) recognized nine valid species; apparently no new forms have been described subsequently. The new worm was studied in comparison with Baer's descriptions of, and found to differ significantly from, all of the old species. Differing from *Cittotaenia sandgroundi* n. sp. with regards to features mentioned, the five previously known avian species are as follows:

(1) *C. africana* Joyeux and Baer, 1927, from *Bucorax* sp. in Africa has the dorsal excretory vessels connected by a transverse canal in each segment, posteriad genital pores, 200 to 240 testes, and paired uteri with the median end of each uterus slightly bifurcate;

(2) *C. avicola* Fuhrmann, 1897, from *Anas* sp. in "Suisse?" has posteriad genital pores and 120 to 140 testes;

(3) *C. kuvaria* (Shipley, 1900) Fuhrmann, 1901 (= *C. columbae* Skrjabin, 1915, according to Baer (1927)), from columbiform birds in Oceania has midlateral genital pores, 100 to 260 testes in 5 to 6 horizontal rows, unarmed cirri, and a uterus with 6 to 8 posteriad diverticula;

(4) *C. psittacea* Fuhrmann, 1904 (= *Paramoniezia suis* Maplestone and Southwell, 1923, according to Baer (1927)), from *Stringops habroptilus* and "*Sus scrofula* L. ???" in New Zealand and Australia has about 200 testes and the uterus located posteriad to testicular field; and

(5) *C. rhea* Fuhrmann, 1904, from *Rhea americana* in South America has about 100 testes, posteriad genital pores, and a diverticulate uterus.

In the description of the family ANOPELOCEPHALIDAE Kholodkowski, 1902, Meggitt (1924), Baylis (1929), Joyeux and Baer (1936), and Craig and Faust (1940)

all describe the scolex as being unarmed and *without a rostellum*. The occurrence of a rostellum in the new species is therefore a very unusual if not unique feature for the family. The small number of testes distinguishes the new worm from all other known species of *Cittotaenia*.

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## EXPLANATION OF PLATE, p. 244

All figures concern *Cittotaenia sandgroundi* n. sp. and were made with the aid of a camera lucida.

## ABBREVIATIONS

a—genital atrium	n—internal seminal vesicles
c—cirrus	o—ovary
d—dorsal excretory canal	s—seminal receptacle
e—ventral excretory canal	t—testes
f—vas deferens	u—uterus
k—external seminal vesicle	v—vagina
m—Mehlis' gland	y—vitellarium

FIG. 1. Mature proglottid; about one-half width of segment represented. Ventral view.

FIG. 2. Portion of partially gravid segment. Ventral view. Testes no longer clearly discernible at this stage.

FIG. 3. Scolex. Dorsal view.

FIG. 4. Gonads, vitellarium, and proximal portions of associated ducts. From a mature segment. Dorsal view.

FIG. 5. Anterolateral corner of a partially gravid segment showing the genital atrium partially everted and forming a collar-like receptacle about the base of the fully protruded cirrus.

FIGS. 6-11. Six sets of gonads showing variations in the arrangement of the testes which are represented in heavy outline. Dorsal views.



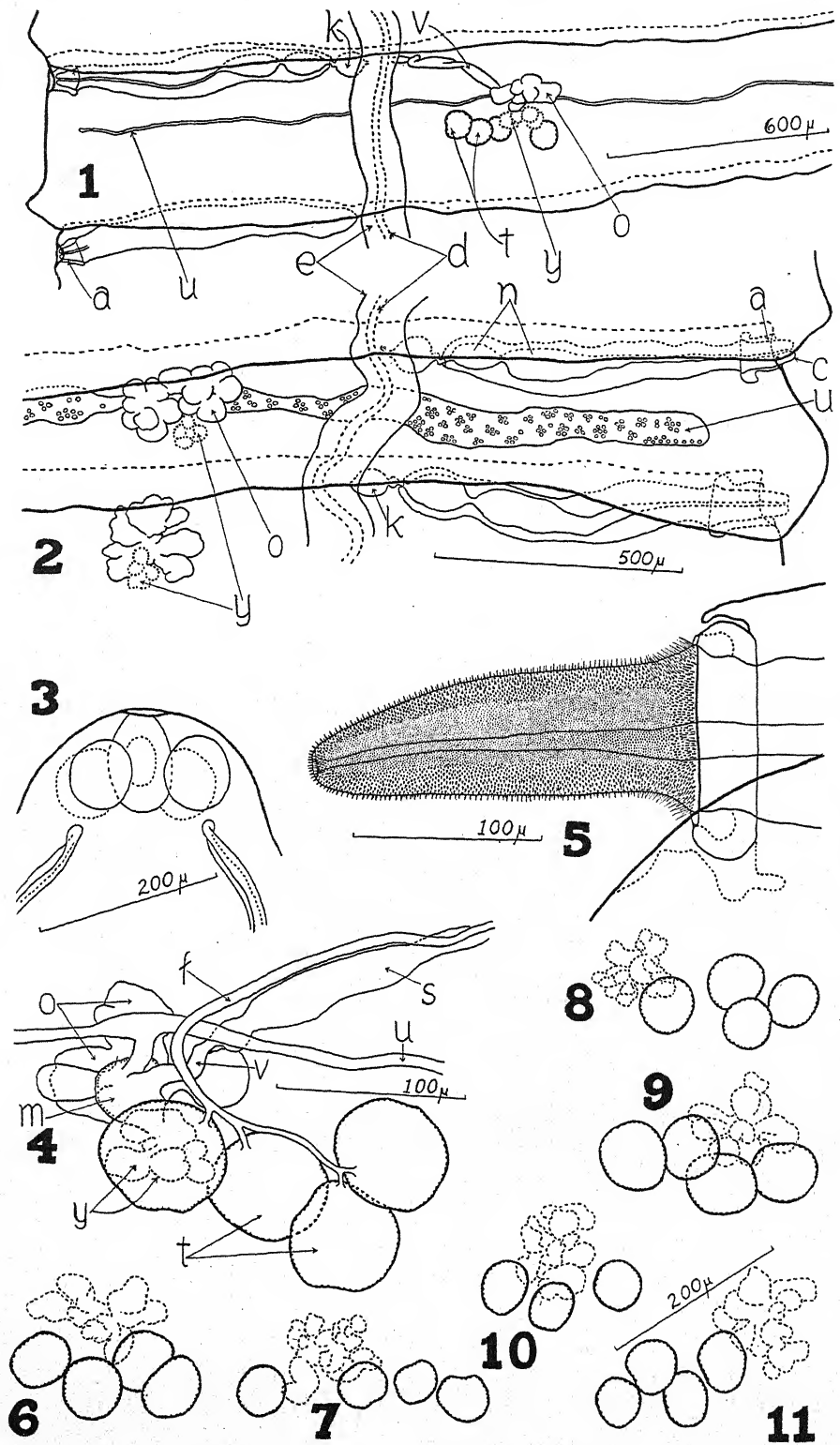


PLATE I

# THE MORPHOLOGY AND LIFE HISTORY OF *FOLEYELLA DUBOISI* WITH REMARKS ON ALLIED FILARIIDS OF AMPHIBIA

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*Rana esculenta ridibunda* from Lake Huleh, North Palestine, is often infected with a filaria which we identify as *Foleyella duboisi* (Geddoelst, 1916) Yorke & Maplestone, 1926. The intensity of infection varies between 3 and 50% in samples brought from different parts of the shore; the cause of this variation is unknown. The adult worms are situated in lymphatic sinuses, mostly in the subvertebral one, or between the serous sheaths of the mesentery. Occasional specimens are found in other sites, i.e., between the lungs, near the anus, etc. Their number in a single frog varies from 1 to 34 but usually 5-6 are found. Females always predominate. Microfilariae are found in the blood, lymph and all tissue fluids and they may easily be observed in the capillaries of the web of the frog's foot stretched under the microscope. Their number apparently depends not only on the number of adult worms present, but also on the duration of the infection. In the early period of infection their number rises to a maximum, but subsequently either larvae are destroyed or the productivity of females diminishes and the microfilariae become less numerous. The presence of only one female may be associated with numerous microfilariae in each drop of blood. The longevity of the adult filariae in frogs kept in captivity is at least one and a half years.

*Foleyella duboisi* has been hitherto recorded only once, Geddoelst (1916), from a "big toad" (crapaud géant) and an undetermined frog in Leopoldville, Belgian Congo, and is rather well described. The examination of our material showed some deviation from the original description and it is therefore advisable to record our observations.

Both males and females are whitish and smooth and move actively when placed in 0.65% saline. The oral opening is round and is guarded by two insignificant lateral folds of cuticle; true lips are absent. There are four small salient papillae situated sublaterally about 20 microns from the oral opening. The latter is continued into an almost cylindrical oesophagus divided into an anterior, short and narrower muscular portion, and a posterior, wider and longer glandular one. The proportion between these two parts is as 1:7 in length. The width of the posterior portion is not uniform, it is slightly narrower in the middle. The part of the intestine which follows the oesophagus is very wide, sometimes almost filling the total width of the body and it gradually narrows posteriorly. On both sides of the anterior extremity, just behind the level of the head papillae, the cuticle forms alae-like fringes, the free edges of which are longer than the line of attachment and therefore the structures are distinctly undulated. These fringes become gradually smoother and narrower and disappear at the level of the beginning of the intestine to reappear again in both males and females in the posterior extremity of the body.

*Male:* Length 9-12 mm, maximum width (0.2 mm) is at the level of the anterior part of the intestine, and the body gradually tapers towards both ends. The oesophagus is 1.4-1.5 mm long. In fixed specimens the posterior extremity of the body is coiled spirally 2-3 times; the ventral

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surface of this part of the body is covered with transverse rows of minute tubercles which disappear about 0.5 mm from the tip of the tail. At this level the lateral fringes reappear and they have the same undulating appearance as at the anterior extremity. They do not reach the tip of the tail and are broadest in their middle. The cloacal opening is situated 0.05–0.08 mm from the tip of the tail. Just at its sides or a little in front, there is a pair of big, semiglobular sessile papillae followed by three pairs of smaller post-anal papillae almost uniform in size. The examination of many specimens has shown that this arrangement is constant. The spicules are conspicuously unequal in size and shape. One is 0.36–0.52 mm long, thin, round in cross section, wider at the proximal extremity. The other is 0.10–0.12 mm long, wide, slightly bent and has a groove on its concave side. The body is expanded by coils of two long and wide seminal vesicles. The extremity of each seminal vesicle terminates abruptly and is continued into a short, club-shaped testis.

*Female:* Length 25–33 mm. The body is almost uniform in width, 0.3–0.5 mm, except at both tapering extremities. The cuticular fringes of the anterior extremity are undulated, as in the male, while at the posterior extremity they are very narrow, smooth, gradually disappearing at about 0.05 mm in front of the tip of the tail. The tail is smooth and has no papillae. Oesophagus is 1.5–1.7 mm long. The posterior part of the intestine widens to form an almost globular ampulla. The anus is situated at ca. 0.4 mm from the tip of the tail. The uterine coils are twisted and expand the body, especially in larger specimens, in which they may reach the posterior extremity; in young specimens they do not reach even the anus. The ovejectors are muscular and much narrower than the uterine tubes. They are long and usually unite in front of the intestine to form the vagina which is 2.0–2.5 mm long, coils round the posterior part of the oesophagus and opens at the level between the second and posterior thirds of the latter.

The above description corresponds to the description and drawing of *F. duboisi* given by Gedoelst (1916) except for the number of oral papillae. Gedoelst mentioned 4 submedian papillae around the mouth, in addition to two lateral ones, while our specimens possess only the former. According to Gedoelst in the species observed by him the lateral fringes commence 0.048 mm from the mouth, i.e., practically at the same distance as the two prominent papillae. We presume that Gedoelst misinterpreted the commencement of the fringes for additional papillae. Fig. 1 made from a preparation of the tip of the anterior extremity of the worm illustrates the possibility of such an error.

Microfilariae of *Foleyella duboisi* may easily be observed in blood vessels of the distended web of a frog's foot. They have an active snake-like movement (Fig. 7); when the blood current is artificially stopped they remain active but hardly change their place. When removed from the blood vessel they wriggle vigorously but do not show translatory movement. In citrated blood they remain active at least two days at room temperature.

In general appearance the microfilariae of *Foleyella duboisi* resemble those of *F. brachyoptera* and *F. ranae* as illustrated by Kotcher (1941). They are slender, almost cylindrical, ca. 5 microns wide at their anterior extremity, tapering gradually to the opposite end. The anterior extremity is obtuse and often forms a knob separated from the rest of the body by a slight constriction. There is a very delicate sheath, longer than the body, visible at one extremity or the other. It is practically unstainable and is best seen in living microfilariae. The cuticle is finely striated. The nuclei are crowded and fill up the entire space between the walls. They are of varying sizes, irregularly round and are all similar with respect to staining properties. In the anterior part of the body they are arranged four abreast, behind the cell  $G_1$  three abreast, behind the cell  $G_4$  two abreast and in the tail there are about 10 nuclei in a single row, the last being often 2–3 times longer than others. The cephalic space is as long as wide, is surrounded by a layer of loose nuclei the disposition of which varies in individual microfilariae, and appears to be devoid of cephalic

structure ("Kopfgebilde"). The nerve ring is a constant feature though it is not always conspicuous (in microfilariae of other species of the genus *Foleyella* it has been reported only in *F. striata*). None of the staining methods used by us revealed a structure corresponding to the central viscus (so called "Innenkörper"). However just behind the middle of the body of the microfilaria there is an unstainable area 5–12 microns long, and adjacent to it one or more ordinary nuclei may be seen in close proximity to the wall. Apparently it is a homologue of the central viscus. The  $G_1$  cell is as wide as the body; other G cells are half this size. The anal-pore cell is situated between the  $G_3$  and  $G_4$ .

The differential characters of the microfilaria of *F. duboisi* are shown on Table 1 together with the characters of other microfilariae of this genus, so far as they are known from the literature.<sup>1</sup> Unfortunately it is impossible to consider other microfilariae of amphibia for the differentiation of species because their descriptions were made from material fixed and stained by methods which do not give uniform pictures. Even dimensions of length are of little value because most of the authors failed to measure the microfilariae in extended condition. If not killed before fixation by heat or other method, microfilariae shrink on the slide during drying to such extent that they are half or less of their original length. Microfilariae of *F. duboisi* killed in fluid by gentle heating of the slide shrink very little. By using Mayer's haematoxylin, Pappenheim's stain, Azur, Giemsa and other stains we were able to establish the fixed cells and spots necessary for differentiation of species. However, staining alone did not make the study of microfilariae simple because their nuclei are thickly crowded and the whole content of a microfilaria absorbs stain so that the nuclear column often appears like a continuous dark mass obscuring the fixed cells. We found that microfilariae taken by a leech remain in its crop not disintegrated and retain staining properties for at least ten days (many of them are motile after 24 hours). During this time the digestion of microfilariae proceeds very slowly and it affects primarily the loose tissue of the latter but not the nuclei. On Giemsa-stained slides made from the gut contents of a leech on the 3d to 10th days after feeding on the frog, the nuclei and the main cells of the microfilariae remain practically unchanged and appear discrete and much more distinct than in untreated material. The microfilariae appear a little swollen, 7–8  $\mu$  wide instead of 5, and their anterior extremity is rounded instead of obtuse. This phenomenon is apparently due not only to slow digestion but also to peculiar preserving properties of the secretion of the leech gut.<sup>2</sup> These results were obtained with an undetermined leech of the genus *Haementeria* and with *Limnatis nilotica* which readily feed on frogs. It is noteworthy that in a dead frog the microfilariae lose their fine structure and after two days no details can usually be made out.

In order to elucidate the life history of *Foleyella duboisi* experiments were performed with mosquitoes, for other species of *Foleyella* are known to be transmitted

<sup>1</sup> We presume that the microfilaria observed by MacFie (1914) in *Bufo regularis* in Nigeria is identical with *Foleyella duboisi*. The approximate formula of this microfilaria, as judged from the drawing of MacFie (No. 2), is as follows: headspace present, NR—12½%, EP—28½%,  $G_1$ —65%,  $G_2$ — $G_4$ —80%. The only difference between this and our microfilaria is the absence of central viscus. However, this may be explained by improper fixation of the slide.

<sup>2</sup> Mathis & Léger (1911: 100) made a similar observation with human spirochaetae. When ingested by a leech, they were found dead after 5 days and showed morphological details very clearly on Giemsa-stained slides.



TABLE 1.—Characters of determinable microflariae of frogs\*

Species	Length in microns	General shape	Cephalic space	in %									
				N.R.	E.P.	E.C.	C.V.	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	A.P.	
<i>brachyptera</i>	128 (Causey, 1939) up to 184 when heat-killed (Kotcher, 1941)	Almost uniform in width Gradually tapering behind C.V.	? absent	23	31	33	65	73	83	85	85	86	
<i>deitchoptera</i>	240 (Causey, 1939) 260-317 when heat-killed (Kotcher, 1941)	Anterior third wider, 2 nuclei abreast, posterior 2/3 narrow, one nucleus abreast	Shorter than width (Causey, 1939) As long as width of the body (Kotcher)	...	19	20	34	55	70	70	71	72	
				12	18	19	31	50	...	...	...	70	
				acc. to Causey, 1939									
<i>duboisi</i>	Heat-killed 200-215, in blood films 95-145.	Uniformly wide up to C.V., afterwards tapers gradually	Always present, though inconsistent in shape	10-23	27-35	29-36	54-68	66-73	75-82	77-83	79-85	78-84	
<i>ranae</i>	90 (Causey) 166-202 heat-killed (Kotcher)	Similar to <i>M. brachyptera</i>	Shorter than width (Causey) absent (Kotcher)	18	27-28	29-31	63-66	71-73	83	85	85-86	88	
<i>ranae-sylvaticae</i>	55-97	Width almost uniform	Contains 2 large nuclei	...	...	26	...	37-60	59-72	62-77	71-86	85-91	

\* Microflaria of *Toxostella neglecta* which is presumably described in the paper of Desportes, 1941, is not included in the table because the original description was not available. All microflariae listed here belong to the genus *Folycyella*, except *M. ranae-sylvaticae* Pantham, Porter & Richardson (1942) of which the adult is still unknown. The characters concerning the latter microflaria have been compiled from two original diagrams.

by these insects. We tried to work with *Anopheles maculipennis* var. *sacharovi* and *A. hyrcanus*, which abound in places where the infection of frogs is common, but these species could not be induced to feed on the frog. *Culex molestus*, which is ubiquitous in Palestine, was then tried with success and all further experiments were done with this species (in Jerusalem, at room temperature, 25–28° C). Some of the mosquitoes were caught in Jerusalem where natural infection of frogs or toads is unknown, and thus infection of mosquitoes could be excluded, but most of them were laboratory bred.

*Culex molestus* does not feed readily on frogs in the laboratory. Some of the insects were induced to bite under an inverted test tube either after being starved for 3–5 days or by raising the temperature of the frog by an electric lamp. Good results were obtained with mosquitoes which were first allowed to bite man, then to lay eggs and to starve 2–3 days. In a few cases the insects were allowed to bite a man for a few seconds and were then placed on a frog on which they continued to feed. Once started, *Culex molestus* feeds avidly. In some cases mosquitoes, gorged on frogs, discharged almost the whole blood meal from the anus a few minutes after feeding.

Twenty per cent of mosquitoes fed on frogs died during the first 24 hours and a further 6% during the following 24 hours. Most of those which survived 48 hours lived up to 20 days. It seems that the number of microfilariae present in the frog's blood influences the mortality of mosquitoes, the heavier the infection the greater the percentage of mosquitoes which die. After the infective feed, the mosquitoes were kept in muslin cages in damp atmosphere and were fed on raisins. In all ca. 250 mosquitoes were used in our experiments. They were examined by dissection or in serial sections between 1–20 days after feeding. About 35% of mosquitoes became infected. Out of 40 mosquitoes dissected 14 days and more after the feed, 18 contained infective stages. In cases of heavy infection of the frog, hundreds of microfilariae may be ingested by the mosquito. However, only a comparatively small number, and in some cases none, penetrate into the haemocoel of the mosquito to continue their development. Microfilariae removed from the mosquito immediately after feeding usually are not as active as in the frog's blood and some may be motionless. In the intestine of dead mosquitoes some microfilariae remain active for 24 hours.

The developmental stages of the larva of *Foleyella duboisi* were followed in mosquitoes dissected at various intervals. It was found that the development proceeds in the haemocoel of the mosquito and only occasional larvae were found between the bundles of the thoracic muscles. The first sign of development may be observed on the third day. The larva becomes shorter, the pointed tail is slightly bent dorsally, the nuclei become swollen and the formation of inner cells is noticeable. On the fourth day the larva reaches the maximum of shortening (115  $\mu$ ), it becomes thicker, the tail is distinctly bent dorsally (Fig. 9). Inside the body a mass of cells is formed, the G-cells are still separated, but the G<sub>4</sub>-cell and the anal cell unite and become attached to the anal slit. A small excretory bladder appears and an indistinct oral apparatus may be visible. On the 5th day the larva (Fig. 10) becomes almost twice as thick at its posterior extremity as at the anterior one, the tail shrinks and the rectum begins to take shape. The latter consists of two big cells and a cavity between them closed by a plug projecting externally. The excretory bladder

is usually distended and lies across the whole width of the body. The inner cells do not as yet show differentiation, except for the big cuticular cells which, especially in the posterior part of the body, are grouped in six loose rows. The above-described larval stage, Figs. 9-10, will be referred to as the tail-bearing stage.

The 6-10-day larva assumes an appearance which we shall call spur-bearing stage (Fig. 11). It reaches a length of 230-300  $\mu$ . The pointed tail has practically disappeared, leaving its cuticle empty, sharply bent dorsally, as a spur-shaped appendage. The inner cells have formed the digestive tube, clearly divided into an anterior longer portion, the oesophagus, and a posterior shorter one, the intestine. The rectum appears like a big bladder and the anus projects externally as a semi-globular papilla. The oral opening projects like a small conical papilla. At this stage the larva shows only very sluggish movement.

After 11-12 days the larva reaches the preinfective stage and prepares to moult (Fig. 12). It is cylindrical, up to 650  $\mu$  long and 40  $\mu$  wide and it moves actively. Both extremities are rounded. The spur-shaped appendage is directed along the axis of the body. The oesophagus and intestine are fully developed, while the rectum is still bladder-shaped and the anus projects externally. The larva is enclosed in a sheath distinctly visible at its extremities.

After 14 days infective larvae may be found (Fig. 13). They are elongated, 640-840  $\mu$  long and 15-22  $\mu$  wide. The anterior extremity bears four minute papillae guarding the oral opening. The tail is more or less elongated, smooth or bears a thickening on the tip. The oesophagus may be slightly shorter than half of the body length. It is wide and is distinctly separated from the intestine. The anus is slightly prominent, 33-40  $\mu$  from the tip of the tail. These larvae are found up to the 20th day and they are scattered in the haemocoel, the head or in the proboscis.<sup>3</sup>

The maximum number of larvae found by us in an individual mosquito was 28. The development does not always proceed at the rate indicated above. It depends on temperature as well as on some other still unknown factors and may be different in individual mosquitoes of the same batch, which were bred, fed and kept under identical conditions. Often larvae in various stages of development are found in the same mosquito, as is shown in the following examples:

*Exp. No. 22.*—7 mosquitoes fed on heavily infected frog. After 15 days 3 proved to be infected. In two of them many infective larvae were found scattered in various parts of the body, only one was in the proboscis. In the third mosquito a few spur-bearing larvae were found in the haemocoel and one motile unchanged (!) microfilaria in the intestine, but no infective larvae.

*Exp. No. 24.*—6 mosquitoes fed on the same frog. After 17 days 3 proved to be infected: (1) 15 infective larvae in the abdomen, thorax and head, 8 spur-bearing larvae and a few intermediate ones in the haemocoel; (2) 3 infective larvae and many spur-bearing ones in the abdomen; (3) 2 infective larvae in the proboscis, 7 in head, abdomen and thorax and a few pre-infective larvae in the haemocoel.

It has to be pointed out that *Culex molestus* is rather rare in the locality where *Foleyella duboisi* is common, and, considering the large percentage of infected frogs, it may be assumed that in nature it does not serve as intermediate host. It is probable that some other mosquito which readily feeds on frogs is a more suitable intermediate host and in it the development proceeds more regularly.

<sup>3</sup> Judging from the observations of Feng (1936) and Kobayasi (1940) on the development of *Wuchereria* and of Highby (1943) on the development of *Dipetalonema* and *Dirofilaria*, it may be assumed that most filariids developing in invertebrates go through four analogous developmental stages of which the first two are commonly included in the general term "sausage-shaped stage."

The life history of *Foleyella duboisi* as interpreted above corresponds to the observations of Causey and Kotcher who in several publications have described the life history of other members of the genus *Foleyella*.

On this occasion it may be remembered that Porta (1912) tried to follow the life history of a frog filaria, which he determined as "*Filaria rubella* Rud.," in the leech, *Hirudo medicinalis*. However, his experiments which are claimed to be positive, were performed in such a manner that the conclusions cannot be accepted. In order to verify the observations of Porta we performed a few experiments in which we let two species of leeches which abound in the locality where *Foleyella duboisi* is common, viz., *Haementeria* sp. and *Limnatis nilotica*, feed on infected frogs. Fourteen days' observation revealed that no migration or development of microfilariae occur. The latter gradually lose their motility during the first 24 hours and remain in the crop of the leech, dead but undigested, sometimes up to 20 days.

TABLE 2.—List of species of the genus *Foleyella*

Name and author	Host	Country
<i>F. americana</i> Walton 1929 .....	<i>Rana pipiens</i>	U.S.A.
<i>F. bouillezi</i> n. nom., 1944 .....	<i>R. stenocephala</i>	U.S.A.
	<i>Bufo regularis</i>	Central Africa
<i>F. brachyoptera</i> Wehr & Causey, 1939 .....	<i>R. pipiens</i>	U.S.A.
	<i>R. sphenocephala</i>	U.S.A.
<i>F. candazei</i> (Fraipont, 1882) Seurat, 1916 .....	<i>Agama colonorum</i>	Africa
	<i>A. stellio</i>	Palestine
	<i>Uromastix acanthinurus</i>	Algiers
	<i>Chameleon</i>	Madagascar
<i>F. chameleonis</i> Kreis, 1938 .....		
<i>F. chlamydosauri</i> (Breinl, 1913) Yorke & Maplestone, 1926 .....	<i>Chlamydosaurus kingi</i>	Australia
<i>F. convoluta</i> (Molin, 1858) Travassos, 1929 (= <i>F. ranae</i> Molin, 1858 nec 1856) .....	<i>Leptodactylus ocellatus</i> , <i>L. pentadactylus</i> , <i>L. typhontus</i> , <i>Hyla faber</i> , ? <i>Bufo marinus</i>	Brazil
<i>F. dolichoptera</i> Wehr & Causey, 1939 .....	<i>R. pipiens</i>	U.S.A.
	<i>R. sphenocephala</i>	U.S.A.
<i>F. duboisi</i> (Geddoelst, 1916) Yorke & Maplestone, 1929 .....	"Big toad," undertermined frog <i>Rana escul.</i> ridib.	Congo
		Palestine
<i>F. furcata</i> Linstow, 1899 nov. comb. ....	<i>Chameleon oustaleti</i>	Madagascar
<i>F. helvetica</i> Kreis, 1934 .....	<i>R. esculenta</i>	Europe
<i>F. leiperi</i> (Railliet, 1916) Yorke & Maplestone, 1926 (= <i>Fil. bufonis</i> Leiper, 1909) .....	<i>Bufo regularis</i>	Sudan
<i>F. ranae</i> Walton, 1929 .....	<i>Rana calamitans</i> , <i>R. catesbiana</i> , <i>R. sphenocephala</i>	U.S.A.
<i>F. scalaris</i> Travassos, 1929 .....	<i>Leptodactylus ocellatus</i>	Brazil
<i>F. striata</i> (Ochoterena & Caballero, 1932) .....	<i>Rana montezumae</i>	Mexico
<i>F. vellardi</i> Travassos, 1929 .....	<i>R. pipiens</i> <i>Bufo marinus</i>	Brazil

THE GENUS *Foleyella* AND ITS SPECIES

Table 2 lists 16 species of the genus *Foleyella*. It was compiled from the literature but the following changes have been considered:

1. *F. bouillezi* nom. nov. is given to an unnamed filaria described by Bouillez (1916) as parasite of *Bufo regularis* in Central Africa. It resembles *F. convoluta* and *F. vellardi* which are insufficiently described. However, the different geographic distribution of the latter (Brazil) suggests that these species may not be identical. According to Railliet (1916) *Foleyella bouillezi* (= *Filaria* sp. of Bouillez) is identical with *Foleyella leiperi*. On the other hand, according to Seurat (1916) the latter is identical with *F. bufonis* of Leboeuf & Ringenbach, 1910. Walton (1935) agreed with this identification, but there seems to be no sufficient proof to support it.



2. We regard *Foleyellides striatus* (Ochoterena & Caballero, 1932) Caballero, 1935 (= *Chandlerella striata*) as a member of the genus *Foleyella* (and therefore the genus *Foleyellides* Caballero, 1935, as superfluous).

3. *Filaria furcata* Linstow, 1899, is regarded as belonging to *Foleyella*.

4. *Foleyella agamae* (Rhodain, 1906) Yorke & Maplestone, 1926, and also the "stumpy type" of microfilaria described by MacFie, 1914, from *Agama colonorum*, are regarded (after examination of Palestinian material from *Agama stellio*) as synonyms of *Foleyella candazei*.

5. *Foleyella helvetica* Kreis, 1934, is left in the list; however, as long as its male is unknown and no lateral cuticular flanges are described, its relation to the genus *Foleyella* remains questionable.

A critical comparison of the known specific characters of the members of the genus *Foleyella* suggests that the conception of the latter needs revision. As a matter of fact, not a single species is fully in accord with the existing diagnoses. According to Yorke & Maplestone (1926), this genus is characterized, *inter alia*, by a mouth surrounded by a circle of six small papillae and four papillae more externally . . . oesophagus very short. . . . According to Skrjabin & Shikhobalova, 1936, the head possesses two lateral papillae and eight submedian ones. . . . We propose, therefore, the following revised diagnosis of the genus *Foleyella* Seurat, 1917:

*Filariinae*: Body smooth or finely striated, or areas on the ventral surface of males in some species covered with small tubercles; mouth without true lips or chitinous or epaulet-like structures, mouth-papillae present or absent; on the sides of the body the cuticle often forms narrow expansions, resembling flanges in some species, which run the whole length of the body or may appear only near the extremities; in the posterior extremity of male these expansions are widened to form caudal alae. *Male*: various numbers of sessile or pedunculated papillae in front or behind the cloacal opening in addition to small papillae at the tip of the tail in some species; spicules very unequal in size and shape: the right one stout and short, the left one considerably longer and thinner; no accessory piece (gubernaculum). *Female*: uteri amphidelph; vulva at the level of the posterior half of the oesophagus or postoesophageal; microfilariae sheathed, in blood. In connective tissue, body cavity or lymphatic sinuses of amphibia and reptiles. *Type species*: *F. candazei* (Fraipont, 1882) Seurat, 1917.

#### OTHER FILARIIDS OF AMPHIBIA

Another filarial genus represented in amphibia is *Icosiella* Seurat, 1917, with the following species:

(1) *I. kobayasii* Yamaguti, 1941, from *Rana limnocharis* in Japan.

(2) *I. neglecta* (Diesing, 1851) Seurat, 1917,<sup>4</sup> recorded from *Rana esculenta* and *R. temporaria* in Europe, from *R. esculenta ridibunda* in North Africa and from *R. tigrina* in Indo-China; vectors *Forcipomyia velox*, *Sycorax siliacea*.

(3) *I. quadrituberculata* (Leidy, 1856) Walton, 1927 (Synonyms: *Filaria amphiumae* Leidy, 1856, *F. nitida* Leidy, 1856, ?*F. Ranae pipientis* "Leidy" of Molin, 1858, *F. solitaria* Leidy, 1856 p.p., *Icosiella solitaria* (Leidy). Recorded from *Rana catesbiana* and *R. pipiens* in U.S.A.

Beside the species of the genus *Foleyella* and *Icosiella* mentioned above, many undetermined or poorly described species of filariae in Amphibia have been recorded in many parts of the world (Table 3). Most of them are probably synonyms of the well-established species. These species inquirendae give additional proof of

<sup>4</sup> Diesing mentioned *Filaria ranae esculentae* Rud. as synonym of this species, apparently owing to common host. As a matter of fact, Rudolphi's species is a *species inquirenda*.

TABLE 3.—List of records of undeterminable or insufficiently described filariae of amphibia

Name and author	Host	Country
<i>F. appendiculata</i> Schneider, 1866	<i>Triturus vulgaris</i>	Europe
<i>F. bufonis</i> M.C.V. Molin, 1858 (= <i>F. Eupemphigis marmorati</i> Molin)	<i>Eupemphigis marmoratus</i>	Brazil
<i>F. cingula</i> Linstow, 1902	<i>Cryptobranchus alleganiensis</i>	U.S.A.
	<i>Megabatrachus japonicus</i>	Japan
<i>F. cochleata</i> Railliet, 1916 (= <i>F. spiralis</i> Oerley, 1882, nec Linstow, 1907)	<i>Helioporus albopunctatus</i>	Australia
<i>F. columbi</i> Blanchard in Montoya y Flores, 1905 (= <i>F. columbia</i> of Linstow, 1907)	Toad	Colombia
<i>F. jubae</i> Seurat, 1916	<i>Bufo bufo</i>	Algeria
<i>F. parva</i> Polonio, 1859	<i>Bufo viridis</i>	Italy
<i>F. ranae</i> Molin, 1856: 391, nec: 390	<i>Hipsiboas faber</i>	Brazil
<i>F. ranae esculentae</i> Rudolphi, 1819	<i>Rana esculenta</i>	Europe
<i>F. ranae esculentae</i> Valentin, 1841	<i>Rana esculenta</i>	Europe
<i>F. ranae esculentae</i> Vogt, 1842	<i>Rana esculenta</i>	Europe
<i>F. rubella</i> Rudolphi, 1819, nec Dujardin	<i>Rana esculenta, R. temporaria</i>	Europe
<i>F. rubella</i> Dujardin, 1845, nec Rudolphi	<i>Rana esculenta</i>	Europe
<i>F. sp.</i> of Baylis, 1933	<i>Rana tigrina</i>	Java
<i>F. sp.</i> of Causey, 1938	<i>Rana oesopus</i>	U.S.A.
<i>F. sp.</i> of Darling, 1912	<i>Bufo marinus</i>	Panama
<i>F. sp.</i> of Franchini	<i>Rana esculenta</i>	Europe
<i>F. sp.</i> of Leger, 1918	<i>Bufo marinus</i>	Fr. Guiana
<i>F. sp.</i> of Leidy, 1852	<i>Rana pipiens</i>	U.S.A.
<i>F. sp.</i> of Leuckart, 1876, of Linstow, 1899, and of Blanchard, 1887	Frogs	Europe
<i>F. sp.</i> of Plimmer, 1912	<i>Bufo marinus</i>	Brazil
<i>F. sp.</i> of Schneider, 1866	<i>Triturus vulgaris</i>	Europe
<i>F. sp.</i> of Schwetzwitz, 1930	<i>Rana alvibraris, Rana occipitalis, Bufo regularis</i>	Congo
<i>F. sp.</i> of Walton, 1927	<i>Amblystoma tigrinum</i>	U.S.A.
<i>F. unguiculata</i> Linstow, 1906	<i>Bufo viridis</i> (Teste Walton)	Europe
<i>Microfilaria bufonis</i> of Leboeuf & Ringenbach, 1910	<i>Bufo regularis</i>	Congo
<i>Microfil.</i> sp. of Mathis & Léger, 1911	<i>Rana limnocharis, R. tigrina</i>	Tonkin
<i>Microfil.</i> sp. of Wenyon, 1931	<i>Rana macrodon</i>	Philippines
<i>Microfil.</i> sp. of Yakimoff, 1916	<i>Rana viridis</i>	Turkestan
<i>Microfil. tamborini</i> Mazza & Franke, 1927	<i>Leptodactylus ocellatus</i>	Brazil
? <i>Spiroptera bufonis</i> Stossich, 1901	<i>Bufo bufo</i>	Sicily

widespread occurrence of filariae in Amphibia. It is, however, noteworthy that in spite of almost cosmopolitan occurrence of filariae in Amphibia their distribution is not uniform. They are confined to some particular localities while they have not been recorded from neighboring ones.

## SUMMARY

*Foleyella duboisi* (Geddoelst, 1916) is common in *Rana esculenta ridibunda* in Lake Huleh, North Palestine. The description is given of adults, microfilaria and developmental stages in experimentally infected *Culex molestus*. The study of partially digested microfilariae taken from the crop of a leech has been found to facilitate the recognition of their peculiar anatomical elements. The development of larvae in the mosquito takes as a rule 14 days but is often irregular. During this development five larval stages are distinguished: 1°—microfilaria, 2°—tail-bearing stage, 3°—spur-bearing stage, 4°—preinfective stage, 5°—infective stage. It is suggested that these stages also occur in other filariae and that the so-called sausage-shaped stage of filarial development includes the above-mentioned stages 2–3. A list of filariid parasites of frogs is given and a revised diagnosis of the genus *Foleyella* is proposed.

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## EXPLANATION OF PLATES

*Foleyella duboisi* (Gedoelst, 1916) Yorke and Maplesstone, 1926

## PLATE I

- FIG. 1. Anterior extremity of an adult specimen, cut obliquely.
- FIG. 2. Anterior end of an adult specimen; scale = 0.04 mm.
- FIG. 3. Anterior extremity of a female.
- FIG. 4. Unfolded posterior extremity of a male.
- FIGS. 5 and 6. Posterior ends of two females with differently developed uteri; scale = 0.1 mm. On Fig. 5, anus is not shown.

## PLATE II

- FIG. 7. Outlines of moving microfilariae.
- FIG. 8. Microfilaria from intestine of leech; scale = 10 microns.
- FIG. 9. Larva; early tail-bearing stage; scale = 10 microns.
- FIG. 10. Larva; typical tail-bearing stage.
- FIG. 11. Larva; spur-bearing stage.
- FIG. 12. Larva; preinfective stage.
- FIG. 13. Infective larva; below, variations of the tip of the tail; scale = 40 microns.

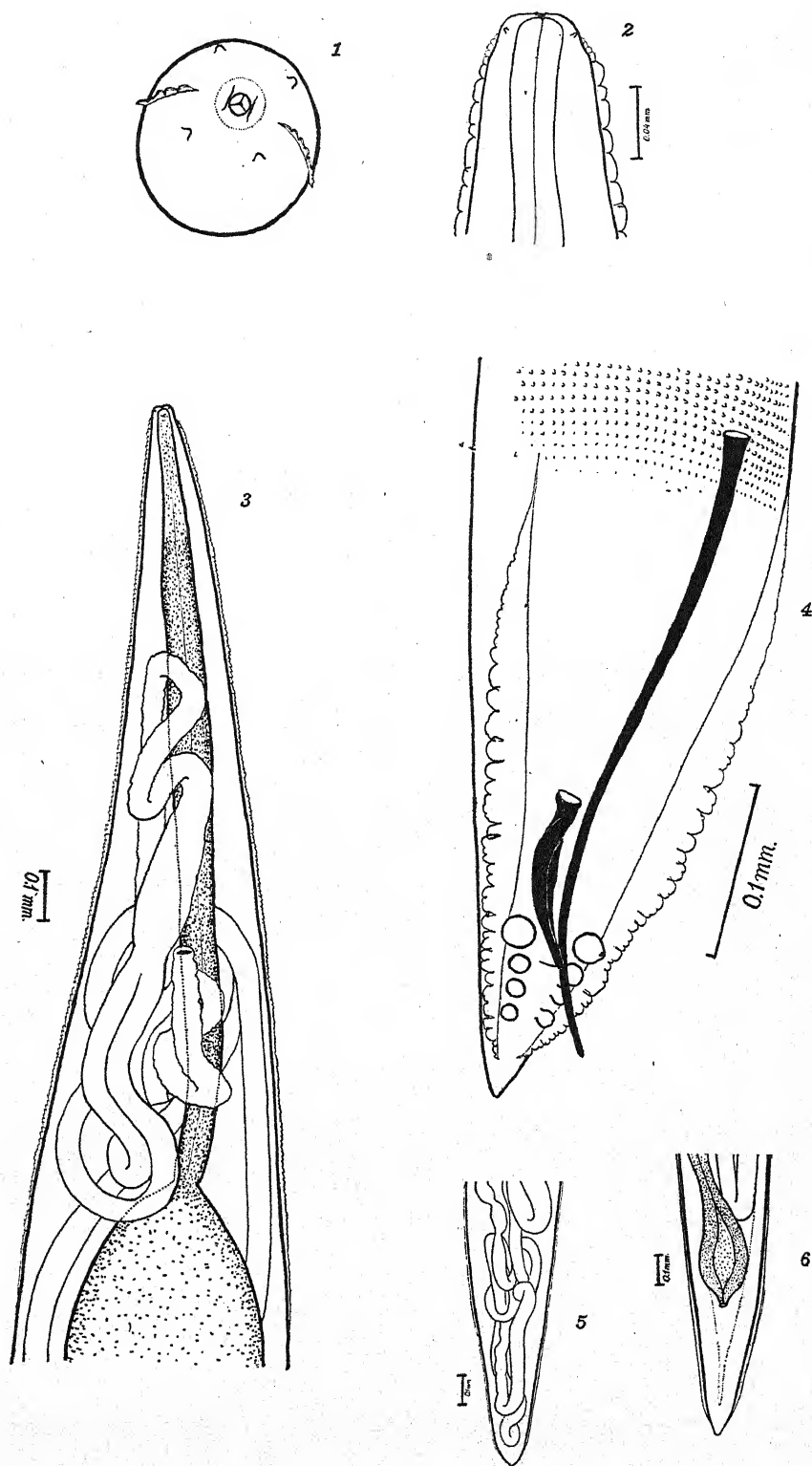


PLATE I



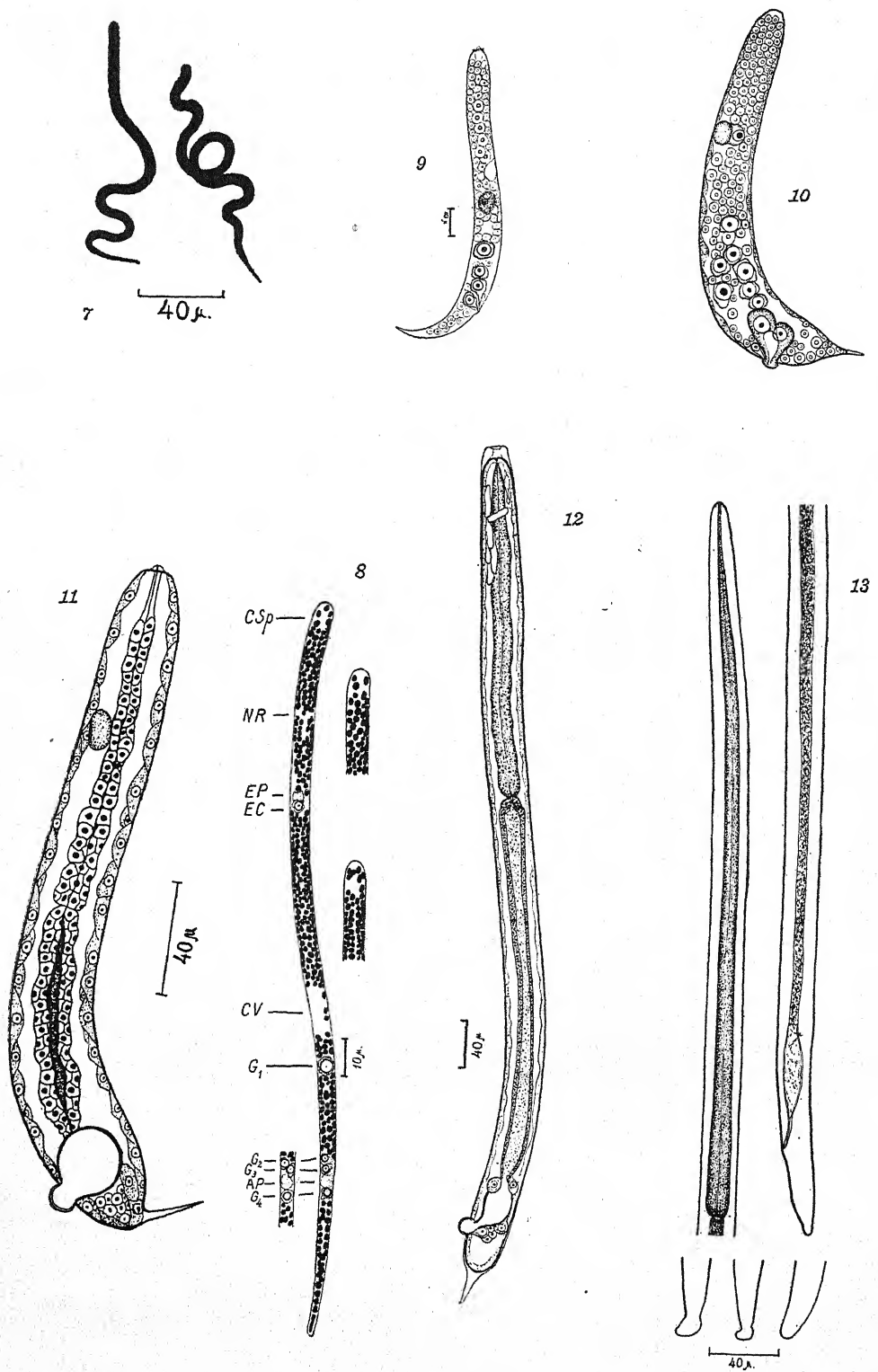


PLATE II

THE LIFE HISTORY OF *NUDACOTYLE NOVICIA* BARKER, 1916  
(TREMATODA: NOTOCOTYLIDAE)\*

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The greater part of the work on the life history of *Nudacotyle novicia* was undertaken at the University of Michigan Biological Station during the summers of 1939 and 1940. Several phases of the study lack completeness, but since further work has not been possible the data at hand are being made available.

Infection experiments involving the feeding of metacercariae of *Cercaria marilli* Ameel, 1939, to various birds and mammals yielded adult specimens of *N. novicia* from the bile duct of the meadow mouse, *Microtus pennsylvanicus pennsylvanicus*. Formerly this species had been reported only from the small intestine of the muskrat, *Ondatra zibethica zibethica*. Subsequently infection experiments with the snail host provided an insight into the early development of this trematode in the intermediate host.

*Daughter redia and cercaria.*—The daughter redia and cercaria, *C. marilli*, from the naturally infected snail host, *Pomatiopsis lapidaria*, have been described previously (Ameel, 1939).

The simple sac-like daughter rediae (Fig. 7) occur in the digestive gland of the snail. The body is pigmented and the mouth is terminal, opening immediately into a muscular pharynx. The gut is moderately voluminous and long, extending to the posterior third of the body. It is easily identified under low magnification because of its contents of reddish brown food particles. There seemed to be a relatively small number of daughter rediae per snail. Of a collection of ten infected snails, the number of rediae recovered ranged from 8 to 39 with an average of 24.

*C. marilli* (Fig. 8) is a trioculate monostome cercaria which occurred in 1.2 per cent of the snails collected at Ann Arbor, Michigan, and in 55 per cent of the snails collected near Marion, Iowa. Both body and tail are capable of considerable extension and contraction. Living cercariae under a supported cover glass measured as follows: body extended, 0.42 mm; contracted, 0.21 mm; tail extended, 0.56 mm; contracted, 0.28 mm. Naturally emerged cercariae have three eyespots but most of the immature cercariae lack the median eyespot. The body is opaque and is filled with brown pigment granules and cystogenous cells packed with rod-like cystogenous material. Spineless posterolateral locomotor organs are present. *C. marilli* resembles the cercaria of *Quinqueserialis quinqueserialis* (Barker and Laughlin) described by Herber (1942). However, the excretory concretions in the excretory bladder of this species are small but numerous, but those of *C. marilli* are large and relatively few.

*The metacercaria.*—When collections of *P. lapidaria* are placed in small bottles or stender dishes containing water, the cercariae emerge in numbers during the evening, night, and early morning, and encyst after a brief period of activity. Some

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\* Contribution from the University of Michigan Biological Station and Contribution No. 238, Department of Zoology, Kansas State College.

cercariae fail to encyst and are found dead upon the bottom of the container. Encystment generally is upon vegetation or the dish, but occasionally cysts are found upon the surface film. If one side of the dish is exposed to light, the cercariae invariably encyst on that side, near the surface of the water. The fact that most of the snails leave the water after a short time or remain partially immersed at the surface indicates that there is a limited period for the emergence of the cercariae after the snails have been placed in the water. Under natural conditions *P. lapidaria* is seldom observed immersed in water but is found living on damp soil near running streams. When fresh collections of these snails were placed in water, there was a good emergence of cercariae overnight, but few cercariae emerged during the succeeding nights. When a continuous supply of metacercariae was desired for infection experiments, the snails were kept on decomposing leaves in shallow dishes. They were removed periodically every week or two and were placed in lettuce cups partially filled with water and fitted into finger bowls in such a manner that when they were covered with glass plates, the snails were sealed in and prevented from wandering far from the water. Large numbers of cysts were collected in this manner. One collection of 558 snails was set out in lettuce cups at 5:30 P.M. Four and one-half hours later the lettuce was thickly covered with cysts, and the snails were removed. Three snails were discovered above the surface of the water surrounded by 34, 57, and 159 cysts, respectively. Evidently the cercariae had emerged in the film of water left by the snails and had encysted immediately. Probably this is the common method of dispersal of the cysts upon the vegetation eaten by the mammalian hosts. It was estimated that this collection of snails yielded over 20,000 cysts.

The cyst is disc-shaped with a flat base (Fig. 9). An elevated portion containing the metacercaria is of smaller diameter than the base. The diameter of the elevated portion of ten unflattened cysts ranged from 0.112 to 0.126 mm (av. 0.117 mm) and that the base from 0.168 to 0.182 mm (av. 0.176 mm).

Feeding experiments have demonstrated that the metacercariae are infective immediately after encystment. Evidently, under proper conditions, they are capable of surviving for a considerable period of time. A collection of approximately 1200 cysts kept in the refrigerator on lettuce for 42 days was fed to a meadow mouse, and 976 young worms were removed from the bile duct upon the death of the animal eight days later.

*Infection of final host.*—Undisturbed cysts on lettuce were fed to three white rats, a pigeon, two rabbits, two guinea pigs, six mallard ducks, two chickens, two muskrats, two canaries, a spotted sandpiper, a bog lemming, and numerous meadow mice. Only the muskrats, meadow mice, and bog lemming, *Synaptomys cooperi cooperi*, became infected.

The adult specimens recovered from the bile duct of meadow mice in most respects resembled *Nudacotyle novicia* common in the small intestine of muskrats from many localities of the lower peninsula of Michigan. That the meadow mouse as well as the muskrat may serve as a natural host of *N. novicia* is indicated from a collection of meadow mice near Marion, Iowa, where the infected snail host is numerous. Two of six trapped meadow mice were infected with this trematode. Three meadow mice trapped in a favorable habitat near Ann Arbor were negative. All the meadow mice collected in the vicinity of the Biological Station have been uninfected.

The bog lemming, which is closely related to the meadow mouse and which shares the same habitat and food, was fed metacercariae to determine whether it could serve as a natural host. The infection was localized in the bile duct. No naturally infected bog lemmings have been collected.

The excysted metacercariae locate exclusively in the bile duct of the meadow mouse within a short time after ingestion of the cysts. The earliest examination of an experimentally infected animal was 18 hours after feeding. After the animal had been fed 300 cysts, 149 worms were recovered from the bile duct and seven from the duodenum. The diameter of the bile duct, which normally is less than a millimeter, increases with the growth of the enclosed trematodes until finally it becomes a conspicuous pink or yellowish sac with a wall so thin that the contents may be discerned through it. The greatest diameter observed was seven millimeters. The average diameter was five millimeters. The usual number of mature worms recovered from an animal surviving the infection was less than 150. As previously mentioned, however, 976 immature worms were removed from the bile duct of an animal that had died eight days after ingesting 1200 cysts. Two hundred and sixty-nine worms with uteri filled with eggs were collected from another animal that had died 17 days after infection. It is assumed that the worms begin to lay eggs at about this age, for a few eggs were first found in routine fecal examinations 18 to 19 days after infection.

The lateral eyespots and scattered patches of body pigmentation are present for some time after the worms become established in the bile duct. All of a collection of 28 nine-day-old worms had scattered remnants of the cercarial pigmentation, but the lateral eyespots remained in 11 specimens. The reproductive system was fully formed but no eggs were present. The pigmentation was more diffuse in 10 eleven-day-old worms, and only one had lateral eyespots. Eggs were observed in 6 specimens; the greatest number in a single specimen being 11. The oldest specimens studied were 64 days old. A marked decline in productivity was evident in these, for the uteri contained but a fraction of the number of eggs observed in worms half their age.

The location of what appeared to be the same species of worm in different parts of the alimentary tract in different but rather closely related hosts caused speculation whether these might be different species. Three wild muskrats one-third to one-half grown were secured through the courtesy of Dr. Lawrence Penner. Two of the muskrats were used as controls. Upon examination, one control was negative, and the other yielded 12 specimens of *N. novicia*. The remaining muskrat, whose feces seemed free of monostome eggs, was fed approximately 600 cysts of *C. marilli*. Four hundred and thirty-seven young adult worms were recovered from the duodenum 28 days later. None was in the bile duct. A trapped adult wild muskrat was fed a total of 2500 cysts over a 12-day period and was examined after the initial feeding. The bile duct was free of trematodes but the duodenum contained 1578. The specimens were in various stages of development, some of the youngest having lateral eyespots. Another adult muskrat caught in the same vicinity as that of the foregoing animal was negative for *N. novicia*. Mature specimens of *N. novicia* from the experimentally infected muskrats in every respect resembled those collected from naturally infected muskrats. Since but two muskrats were fed experimentally and only wild-caught animals were used, the results of this experiment are not regarded



as conclusive. The evidence, however, is sufficient to indicate that *C. marilli* probably develops into *N. novicia* in either the bile duct of the meadow mouse or in the duodenum of the muskrat.

*The adult.*—Specimens from the meadow mouse (Fig. 1) are on the average considerably larger than those from the muskrat (Fig. 2). Fifteen worms chosen at random from a collection from a meadow mouse measured 0.903 to 1.407 mm (av. 1.123 mm) in length and from 0.462 to 0.833 mm (av. 0.615 mm) in width. Ten specimens from a naturally infected muskrat measured 0.585 to 0.735 mm (av. 0.661 mm) in length and 0.315 to 0.45 mm (av. 0.376 mm) in width. Barker (1916) described *N. novicia* from material from the muskrat. The lengths of his specimens ranged from 0.709 to 0.899 mm, and the widths from 0.501 to 0.657 mm.

Specimens of *N. novicia* from the meadow mouse generally are plump with tapered posterior ends, but those from the muskrat are typically thin dorso-ventrally with concave ventral surfaces and truncate posterior ends.

Since *N. novicia* has been collected from muskrats in the vicinity of the Biological Station and since the known snail host, *P. lapidaria*, has never been reported from the region in spite of extensive collecting, indications are that some other species of snail must also serve as intermediate host. Large collections of local snails have been examined, but none has been found infected with *C. marilli*.

*The egg.*—Within three to four weeks after infection of the host, numerous eggs appeared in the feces. On several occasions, upon examination of the host, great numbers of eggs were recovered from the bile duct, where possibly they had been trapped.

The eggs (Fig. 3) are amber colored and operculate and bear long polar filaments. Occasionally, atypical eggs with double abopercular filaments were observed in fecal smears (Fig. 4). Barley perceptible miracidia are present in the eggs which have passed from the host.

Fifteen eggs measured in a fecal smear had lengths ranging from 0.019 to 0.022 mm (av. 0.021 mm) and widths from 0.013 to 0.016 mm (av. 0.015 mm). The full length of the egg including the filaments varied from 0.469 to 0.536 mm (av. 0.503 mm).

At two different times eggs were kept in watch glasses at room temperature for a month and a half but none was observed to hatch. It is assumed that possibly they must be eaten by the proper snail host before hatching occurs, though Herber (1942) in his life history studies of two monostomes stated that the miracidia probably hatch and actively penetrate the host. On one occasion a snail was observed to include an egg while ingesting debris from the bottom of the dish.

*Infection of snails in laboratory.*—Since nothing was known about the rearing of *P. lapidaria* in the laboratory, it was necessary to obtain young snails from a natural habitat and to maintain controls. This species of snail has never been found in the northern half of the Lower Peninsula of Michigan, and so it was necessary to secure young snails from Ann Arbor. On August 3, 1940, fifty young snails of about one to one-and-one-half millimeters in length were placed in uncovered large stender dishes containing moist soil and decomposing leaves to which were added a generous number of eggs collected from adult worms raised experimentally in the meadow mouse. For some unknown reason most of the snails died within a week. No sign of infection could be found in the few survivors examined at this

time. None of 26 surviving controls was infected. Since it was too late for further work at the Biological Station, a supply of eggs and six recently infected meadow mice were taken to Manhattan, Kansas, and another collection of young snails was made at Ann Arbor enroute. The snails were kept in flat pans on damp sod removed from their habitat. Decomposing leaves were added to the pans from time to time.

On October 2, 1940, the mice were sacrificed and eggs recovered. The procedure that followed was identical with that used in the first infection experiment. Since the snails had been in the laboratory for over a month, 50 regarded as controls were examined at the onset of the experiment. Of this number only one was infected.

*Mother sporocyst.*—Seven days after exposure three snails were examined, but nothing was found that could be positively identified as a mother sporocyst. When two snails were examined seven days later, one mother sporocyst was recovered from one, and two from the other. Over the following week, further examinations yielded more mother sporocysts. In every case, these were found loosely attached along the intestine or floating free in the dish. Only one living mother sporocyst was studied. The remainder was fixed in Gilson's, then stained, and mounted for microscopic examination. Unfortunately but three were satisfactory for study. These measured respectively: 0.130 by 0.087 mm, 0.119 by 0.098 mm, and 0.112 by 0.093 mm. Two mother sporocysts contained four developing mother rediae (Fig. 5), but the other contained five.

*Mother redia.*—After three weeks, every snail examined yielded rediae in some stage of development. These were located either along the intestine anterior to the digestive gland or in the digestive gland. Examination of snails continued until November 18, 1940, when the remaining snails were examined since they were in poor condition. A total of 39 snails was examined of which three possibly were negative. No doubt, because of low temperatures, development had lagged, and no daughter rediae that could be identified positively as such were observed. Some of those recovered toward the latter part of the experiment may have been young daughter rediae. Unfortunately, the material obtained from the experimental infection was such that a careful study of the early generations in the snail was not possible. Further work on this phase of the life cycle is desirable.

The gut of the mother redia (Fig. 6) was readily observed under low magnification, for it was brownish or amber-colored due to its contents of food particles and was relatively large, being globular or elongate in shape. The number of fully formed daughters within any mother redia generally were few. The measurements of 10 fixed and stained mother rediae collected four weeks after infection were 0.14 to 0.248 mm (av. 0.19 mm) in length and 0.084 to 0.109 mm (av. 0.094 mm) in width.

#### SUMMARY

The life cycle of *Nudacotyle novicia* has been completed in the laboratory by the feeding of encysted *Cercaria marilli* to wild and laboratory raised animals.

The snail host is *Pomatiopsis lapidaria*. The definitive hosts are the meadow mouse and the muskrat. The adult trematode lives in the bile duct of the meadow mouse and in the small intestine of the muskrat.

Mother sporocysts and mother rediae were recovered from young *P. lapidaria* exposed to the eggs of *N. novicia*. These forms are described.

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## EXPLANATION OF PLATE I

(All drawings made with aid of camera lucida)

- FIG. 1. An adult specimen from bile duct of meadow mouse.
- FIG. 2. Outline of adult specimen from duodenum of muskrat.
- FIG. 3. Normal egg.
- FIG. 4. Egg with two abopercular filaments.
- FIG. 5. Mother sporocyst.
- FIG. 6. Mother redia.
- FIG. 7. Daughter redia.
- FIG. 8. Mature cercaria.
- FIG. 9. Encysted metacercaria.

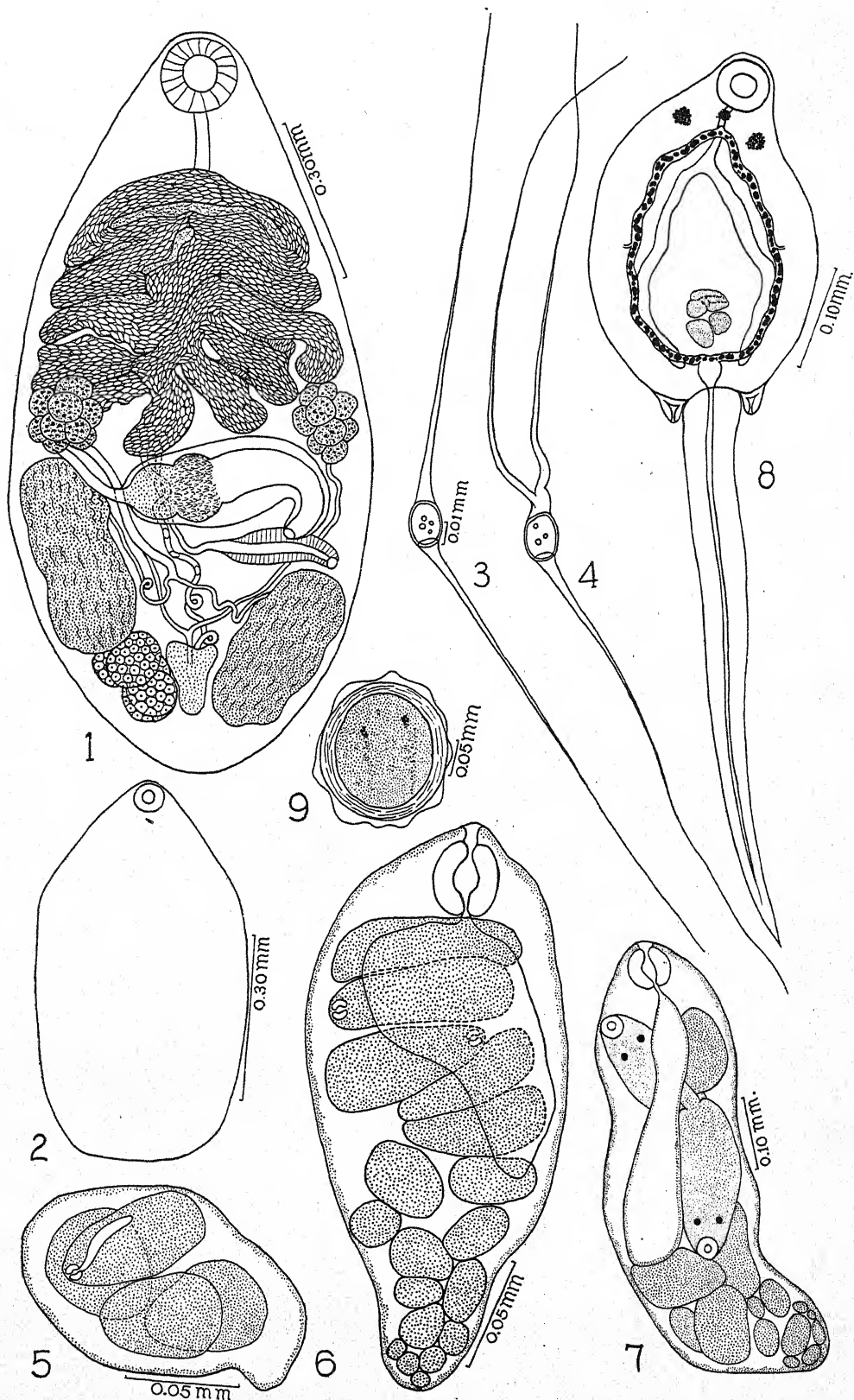


PLATE I



# STUDIES ON *CERCARIA SZIDATI* SP. NOV., A NEW FURCOCERCIOUS CERCARIA OF THE VIVAX TYPE

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## INTRODUCTION

A new longifurcous, pharyngeate cercaria of the Vivax type has been found to develop in *Campeloma* sp. collected from the Tippecanoe River, near Lafayette, Indiana. Although about fifteen species of Vivax larvae have been described, *C. kentuckiensis* Cable, 1935, has been the only fresh water representative of the group reported from the United States. Cable (1938) extended his brief description of this species and gave a comprehensive review of the group in a paper to which the reader is referred. The present species is named in honor of Professor L. Szidat who found a very similar cercaria in East Prussia and determined it to be the larva of *Linstowiella viviparae* (von Linstow, 1877).

Snails were collected during the summers of 1942-43 and examined for emerging cercariae which were studied alive, both unstained and with the aid of supra-vital dyes. Measurements were taken from larvae killed in hot 10% formalin because living cercariae were so active that they could not be measured without excessive cover-glass pressure. A few infected mollusks were crushed to obtain sporocysts and observe the development of cercariae. This investigation was carried out under the direction of Dr. R. M. Cable, whom I wish to thank for his interest, criticism, and suggestions.

## OBSERVATIONS

Description of *Cercaria szidati* sp. nov.

(Figs. 1 and 2)

*Specific diagnosis:* Longifurcous pharyngeate cercaria with the characters of the Vivax group. Measurements in mm of ten specimens: body length 0.386-0.495 (av. 0.437), width 0.172-0.248 (0.205); length of tail-stem 0.694-0.819 (0.742), width 0.086-0.106 (0.098); length of tail furcae 0.429-0.532 (0.471); length of oral sucker 0.076-0.093 (0.087), prepharynx 0.007-0.013 (0.012), and pharynx 0.02-0.033 (0.027). Body pyriform, flattened, and spinose except ventral surface of posterior body region. Tail attached dorsally; tail-stem and furcae spined and with delicate hair-like processes; furcal fin-folds absent. Intestine prominent, with tortuous ceca extending to level of excretory vesicle. Excretory system typical of the Vivax group, the vesicle receiving two median and two lateral ascending tubules; median tubules converge anterior to a prominent mass of nuclei (probably the primordium of the holdfast organ), and fuse to form a single median tubule which extends anteriorly to join a cross-commissure connecting the pair of lateral ascending tubules. Slightly posterior to this level, each lateral tubule receives the main collecting tubule which extends posteriorly a short distance before dividing into anterior and posterior secondary tubules. Each anterior tubule receives the capillaries of two groups of three flame cells; each posterior tubule is joined by the capillaries of three similar groups and extends into the tail-stem where it receives an additional group of three flame cells; the excretory formula accordingly is  $2[(3+3) + (3+3+3+3)]$ . Develop in sausage-shaped sporocysts in the mantle region of the molluscan host.

*Host:* *Campeloma* sp.

*Locality:* Tippecanoe River, Indiana, U.S.A.

*Incidence of infection:* 8.0%.

The cercaria exhibits no decided phototropism and does not react to shadows. However, it responds instantly to the slightest movement of the water by swimming

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in a rapid, jerky manner and then stops suddenly at or near the surface. The larva spends much of the time at rest with the tail flexed, usually to the right, so that the distal part of the tail-stem is about perpendicular to the body axis. Before death, cercariae frequently creep over the bottom of the dish with rapid inchworm movements, occasionally lashing the tail violently, but not rising. Decaudation is frequently observed, and shortly after separating from the body, the tail becomes vacuolated and contracts with the furcae rolled into a tight spiral. The larva will attempt to penetrate anything in its path, often forcing its way into vaseline sealing the preparation. After emergence, the cercariae live from 12 to 24 hours, rarely longer.

Neutral red has a selective action on the glands in the penetration organ and fore-body, the intestinal ceca, and the Island of Cort, while the nuclei of the body and tail are less intensely stained.

Only sporocysts producing cercariae have been observed in the present study (Fig. 2). They are provided with characteristic circular muscle bands and move very actively when dissected from the snail. Unlike the sporocysts of *C. kentuckiensis* which occur in the digestive gland of the snail, those of *C. szidati* are found only in the mantle region and adjacent portions of the visceral mass. The excretory pattern of the sporocyst was not determined, but enough was observed to show that it was very complex.

*Cercaria kentuckiensis*, or a very similar species, occurs in *Goniobasis depygis* from McCormicks Creek, Indiana, and has been available for comparison with *C. szidati*. These species are similar in respect to body shape and spination and swimming activities. In both, the muscle fibers of the tail-stem have a distinctly striated appearance under high magnification, and the tail-stem and furcae are spinose, although Cable (1938) did not find spines on the tail-stem of *C. kentuckiensis*. These species differ in many respects, however. *C. szidati* is much the larger of the two, lacks furcal fin-folds, and has less tortuous intestinal ceca which, furthermore, contain no concretions resembling those observed in the intestine of *C. kentuckiensis*. Also, the body of *C. szidati* is less glandular, especially in the lateral and posterior regions, making it much easier to trace the flame cell pattern than in *C. kentuckiensis*. Although Cable (1938) found 14 pairs of flame cells in the latter species, he did not observe the capillaries and their connections. Since the flame cells of *C. kentuckiensis* are much smaller than those of *C. szidati* and are extremely difficult to observe, it seems probable that both species may have the same pattern, viz., 15 pairs of flame cells in the body. Although the flame cell pattern may be identical in these species, several differences in their excretory systems are apparent upon careful study. The median and lateral ascending tubules of *C. szidati* lack concretions, and the anterior ends of those tubules are not extended as in *C. kentuckiensis*. Also, the median pair forms a single loop embracing the primordium immediately anterior to the excretory vesicle, instead of two loops as in *C. kentuckiensis*. The primordium of the ventral sucker, which is enclosed by one loop in the latter species, appears to be undeveloped in *C. szidati*. Another difference in the excretory system of the two species is provided by the furcal tubules which are always distinct in *C. szidati* and situated near the middle of the furcae. Pores at the tips of the furcae were observed in immature specimens, but could not be demonstrated in larvae emerging from the snail.

Of the reported species of cercariae belonging to the Vivax group, *C. szidati* resembles most the cercaria of *Linstowiella viviparae* (von Linstow) as described by Szidat (1933). However, these species differ in respect to body size and the proportionate lengths of the tail-stem and furcae, and their excretory patterns are quite dissimilar. According to Szidat, the larva of *L. viviparae* penetrates and encysts in the Molluscan intermediate host. In the present study, specimens of *Campeloma* sp., both infected and uninfected, and other species of snails were exposed to *C. szidati* for prolonged periods, but no metacercariae were recovered in subsequent examinations. This failure to encyst in the molluscan host provides an additional difference between *C. szidati* and the larva of *L. viviparae*.

Sewell (1922) divided the Vivax group of furcocercariae into two subgroups, and subsequent investigators (Faust, 1922; Szidat, 1933) have proposed additional subgroups on the basis of excretory patterns and tail structure. Cable (1938) re-defined and simplified the classification to some extent, accepting three of the subgroups previously proposed, viz., the Vivax, Tetis, and Tauiana Subgroups. In defining the Vivax subgroup, he overlooked the fact that the cercaria of *L. viviparae* lacks furcal fin-folds and a recognizable ventral sucker. By possessing three pairs of flame cells in the tail-stem, and lacking a ventral sucker and furcal fin-folds, both *C. szidati* and the larva of *L. viviparae* overlap the Vivax and Tetis subgroups. It therefore becomes necessary to combine these subgroups and recognize at the most, only two subgroups of Vivax furcocercariae: (1) the Vivax subgroup which includes those with flame cells in the tail-stem and (2) the Tauiana subgroup whose members lack such flame cells. Indeed, further studies on life histories may demonstrate that even this distinction is of minor importance, and may warrant further simplification, even to the extent of combining the Vivax groups with other types of holostome cercariae. This would be warranted if further studies should reveal that the complex nature of the ascending excretory tubules in Vivax cercariae represents merely a precocious development of the so-called "reserve system" characteristic of the strigeids.

#### SUMMARY

*Cercaria szidati* sp. nov. is reported from *Campeloma* sp. collected from the Tippecanoe River, Indiana, U.S.A. The species is described and compared with other furcocercariae of the Vivax group and the classification of the group is discussed.

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## EXPLANATION OF PLATE, p. 268

All figures pertain to *Cercaria szidati*

## ABBREVIATIONS

ce	cercarial embryo	in	intestine
cg	cephalic glands	le	lateral excretory tubule
ct	caudal excretory tubule	mb	muscle band
ev	excretory vesicle	me	median excretory tubule
ft	furcal excretory tubule	os	oral sucker
hf	primordium of holdfast organ	ph	pharynx
ic	Island of Cort		

FIG. 1. Cercaria, ventral view.

FIG. 2. Sporocyst.



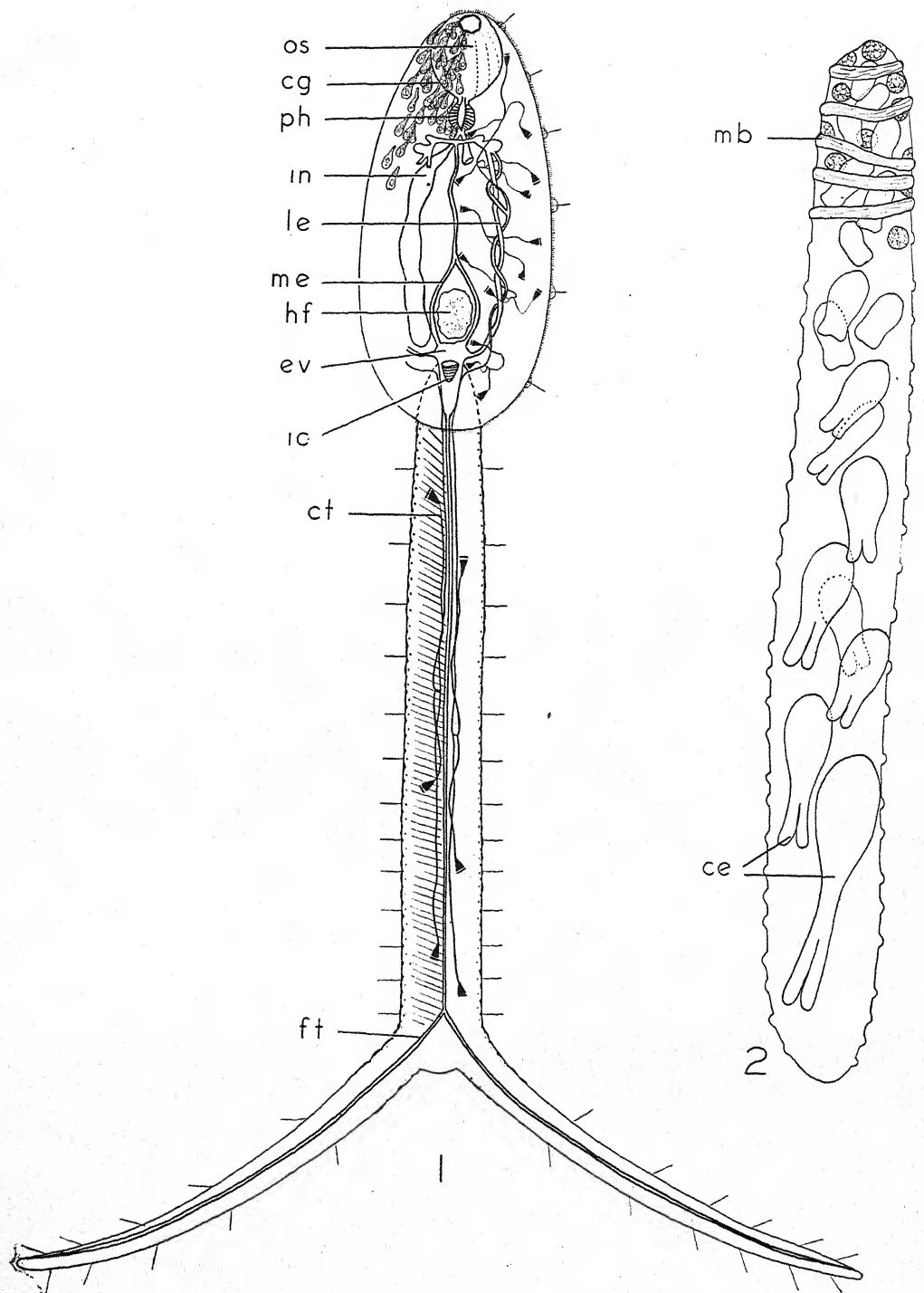


PLATE I

## TWO NEW NEMATODES FROM THE AQUATIC BEETLE *HYDROUS TRIANGULARIS* (SAY)<sup>1</sup>

A. C. TODD

Louisiana State University

The worms described below were recovered from specimens of the large water scavenger beetle, *Hydrous triangularis* (Say), taken in Nebraska and Louisiana. The new nematodes belong to the family *Thelastomatidae* Travassos, 1929 and to the subfamily *Thelastomatinae* Travassos, 1920. They have been placed in the genus *Pseudonymus* Diesing, 1857 and perhaps constitute the first record of the genus from the United States.

*Pseudonymus brachycercus* n. sp.

(Measurements in millimeters)

Figs. 1-3

With the characters of the genus as given by Diesing (1857).

*Male*: Unknown.

*Female*: 3.15-4.11 long by 0.218-0.295 wide. Mouth opening prismoidal and surrounded by a circumoral elevation which bears eight papillae submedially arranged. Lateral organs or amphids present. Second annule enlarged, 0.015-0.025 long, forming a distinctive cephalic ring. Esophagus 0.43-0.50 long, consisting of an anterior cylindrical portion 0.328-0.385 long by 0.0375-0.0475 (at nerve ring) wide, followed by a short isthmus 0.0125-0.02 long and terminating in a valvular bulb 0.0825-0.103 long by 0.095-0.113 wide. Nerve ring 0.233-0.295 from anterior end of esophagus. Excretory pore elevated, posterior to base of esophagus, 0.52-0.65 from anterior end of body. Anterior end of intestine somewhat dilated; anus 0.361-0.438 from posterior end of body. Tail bluntly attenuated. Reproductive system amphidelphic. Vulva 1.772-2.616 from anterior end of body; lips not salient. Ovejector directed somewhat laterally; uteri bifurcating near middle of body. Eggs oval, 0.078-0.088 long by 0.048-0.055 wide, ensheathed in a spiral filament and containing a fully formed embryo when deposited.

*Host*: *Hydrous triangularis* (Say).

*Location*: Large intestine.

*Distribution*: U.S.A. (Nebraska, Louisiana).

*Holotype*: Female, U.S.N.M. Helm. Coll., 36896; *Paratype*: Female, No. 36896.

*Pseudonymus leptocercus* n. sp.

(Measurements in millimeters)

Figs. 4-6

*Male*: Unknown.

*Female*: 2.47-2.94 long by 0.145-0.22 wide. Mouth opening prismoidal and surrounded by a circumoral elevation which bears eight papillae submedially arranged. Lateral organs or amphids present. Second annule enlarged, 0.015-0.017 in length, forming a cephalic ring. Esophagus 0.385-0.423 long, consisting of an anterior cylindrical portion 0.293-0.33 long by 0.035-0.037 (at nerve ring) wide, followed by a short isthmus 0.017-0.02 long and terminating in a valvular bulb 0.065-0.08 long by 0.075-0.085 wide. Nerve ring 0.2-0.25 from anterior end of esophagus. Excretory pore elevated, posterior to base of esophagus, 0.484-0.546 from anterior end of body. Anterior portion of intestine somewhat dilated; anus 0.381-0.463 from posterior end of body. Tail filiform. Reproductive system amphidelphic. Vulva 1.450-1.710 from anterior end of body; lips not salient. Ovejector directed laterally; uteri bifurcating near middle of body. Eggs oval, 0.078-0.088 long by 0.048-0.055 wide, ensheathed in a spiral filament and containing a fully formed embryo when deposited.

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<sup>1</sup> Contribution No. 65 from the Dept. of Zoology, Entomology and Physiology, Louisiana State University.

Host: *Hydrous triangularis* (Say).

Location: Large intestine.

Distribution: U.S.A. (Nebraska, Louisiana).

Holotype: Female, U.S.N.M. Helm. Coll., No. 36897; Paratype: Female, No. 36897.

#### DISCUSSION

The genus *Pseudonymus* was erected by Diesing (1857) to receive *Oxyuris spirotheca* which had been described by Györy in 1856 from the aquatic beetle, *Hydrophilus piceus*; the worm then became *Pseudonymus spirotheca* (Györy, 1856) Diesing, 1857. Galeb (1878) described and figured four species of nematodes from hydrophilid beetles, among which was *Oxyuris (Helicothrix) spirotheca* from the beetle *Hydrophilus piceus*; he erected the subgenus *Helicothrix* to accommodate the four worms. Stiles and Hassall (1905) indicate *Helicothrix* to be a synonym of *Pseudonymus* and designate *P. spirotheca* as the type of its genus.

Basir (1941) established the genus *Galebiella* for two worms, *G. galebiella* and *G. istamabadi*, from aquatic beetles in India. He distinguished his genus *Galebiella* from the genus *Leidynemella* Chitwood, 1934 but did not refer to the genus *Pseudonymus*. The genus *Galebiella* agrees with the genus *Pseudonymus* noticeably in that both possess enlarged cephalic annules and in other morphological characters; they both are parasitic in aquatic beetles. *G. istamabadi* appears closely related to *P. spirotheca* which has been designated as a type species as mentioned above. From Basir's descriptions and figures *Galebiella* would appear to differ from *Pseudonymus* in that its eggs are not embryonated when deposited and are not ensheathed in a spiral filament. The tails of *G. galebiella* and *G. istamabadi* are conical and Galeb indicated that the tail of *P. spirotheca* was similar, among others, to that of *Hammerschmidtella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 and *Leidynema appendiculatum* (Leidy, 1850) Chitwood, 1932 which do not have conical tails.

The genus *Zonothrix* Todd, 1942 was established by the present writer for *Z. tropisterna* a parasite of a water scavenger beetle, *Tropisternus nimbatus*. *Oxyuris (Helicothrix) hydroi* Galeb, 1878 was renamed *Zonothrix hydroi* (Galeb, 1878) Todd, 1942. The genus *Zonothrix* was separated from its closely related genus, *Pseudonymus*, by the absence of cephalic rings, the location of the vulva at the posterior third of the body and the prolonged condition of the anterior lip of the vulva.

Of the two new worms, *P. brachycercus* appears to resemble *Pseudonymus hydrophili* (Galeb, 1878). The length of the female *P. hydrophili* is given by Galeb as 4 mm which is within the limits of the length of *P. brachycercus*, i.e., 3.15–4.11 mm. The two worms cannot be further distinguished because a full description of *P. hydrophili* was not given. *P. leptocercus* can be distinguished from *P. brachycercus* by its smaller size (2.47–2.94 mm compared to 3.15–4.11 mm) and because it has a filiform tail.

#### SUMMARY

1. The genus *Pseudonymus* Diesing, 1857 has been recorded from water scavenger beetles in the United States.
2. Two new parasitic nematodes, *Pseudonymus brachycercus* and *P. leptocercus* have been described from the water scavenger beetle, *Hydrous triangularis* (Say), in Nebraska and Louisiana.

3. The genus *Galebiella* Basir, 1941 has been related to the genus *Pseudonymus* Diesing, 1857.

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## EXPLANATION OF PLATE, p. 272

- |   |   |
|---|---|
| FIG. 1. <i>Pseudonymus brachycercus</i> . Head of female, en face view. | FIG. 4. <i>P. leptocercus</i> . Head of female, en face view. |
| FIG. 2. <i>P. brachycercus</i> . Adult female.                          | FIG. 5. <i>P. leptocercus</i> . Adult female.                 |
| FIG. 3. <i>P. brachycercus</i> . Egg.                                   | FIG. 6. <i>P. leptocercus</i> . Egg.                          |

All drawings were made with the aid of a camera lucida.



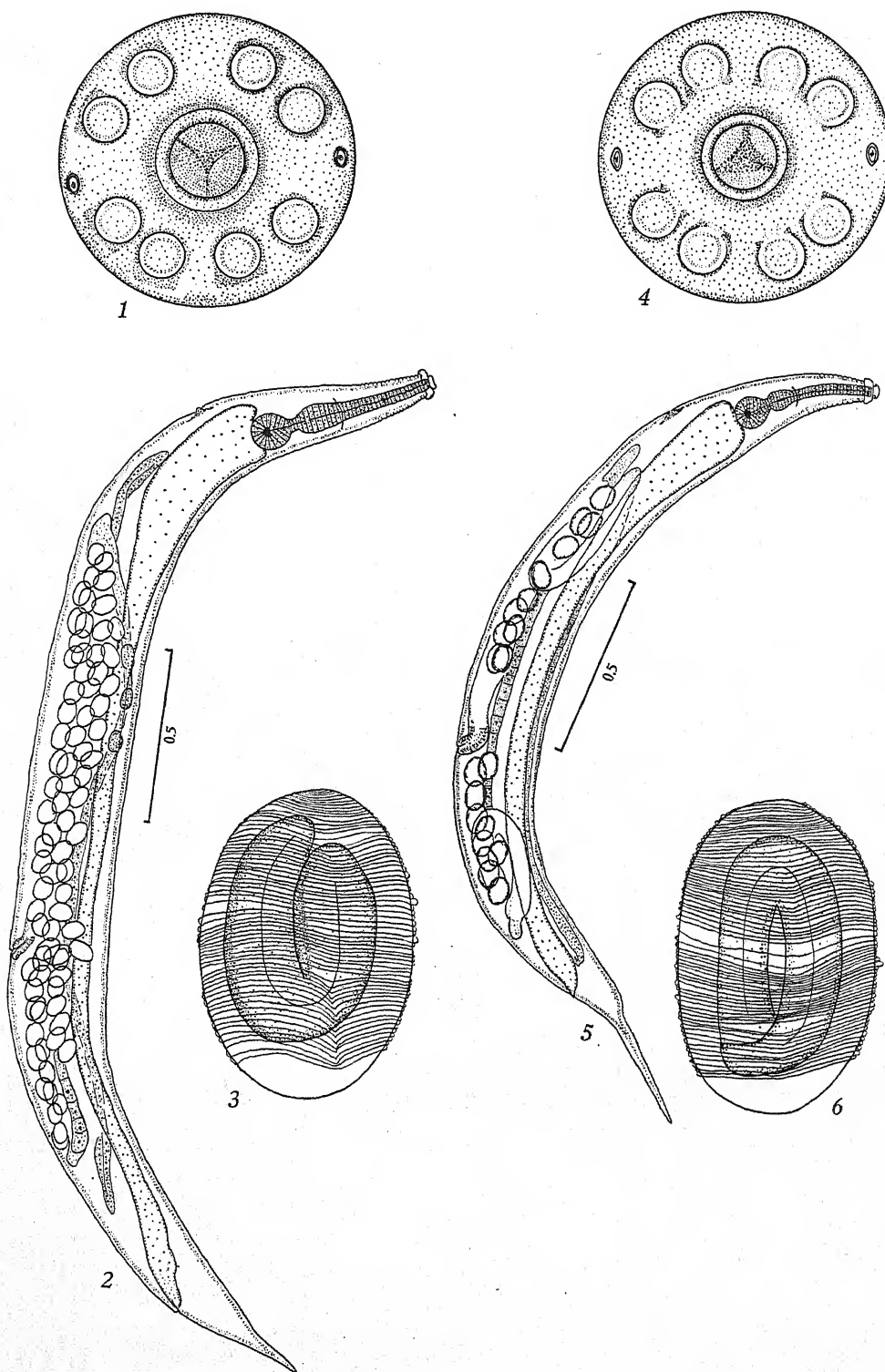


PLATE I

## RESEARCH NOTES

### A NEW SPECIES OF *MESOCESTOIDES*, *M. KIRBYI*, FROM *CANIS LATRANS*

Several lots of *Mesocestoides* collected from *Canis latrans* in California, and sent to the writer for identification, differ from species of this genus hitherto described in several respects, and are therefore considered a new species, for which the name *Mesocestoides kirbyi* is proposed.

Diagnosis: Length of strobila 19 to 30 cm, maximum width 1.7 to 2 mm. Scolex 350 to 470  $\mu$  wide and 360 to 440  $\mu$  long. Suckers 230 to 300  $\mu$  long and 130 to 150  $\mu$  wide, with slit-like openings, 170 to 200  $\mu$  long. Calcareous corpuscles few and scattered. Mature segments nearly as long as wide when relaxed. Gravid segments strikingly bell-shaped, 2.2 to 4 mm long and 1.1 to 1.5 mm wide, junction with anterior segment only 200 to 500  $\mu$  wide. Testes 100 to 120 in number, 45 to 65  $\mu$  in diameter. Cirrus pouch 180 to 210  $\mu$  long and 130 to 150  $\mu$  wide. Ovaries

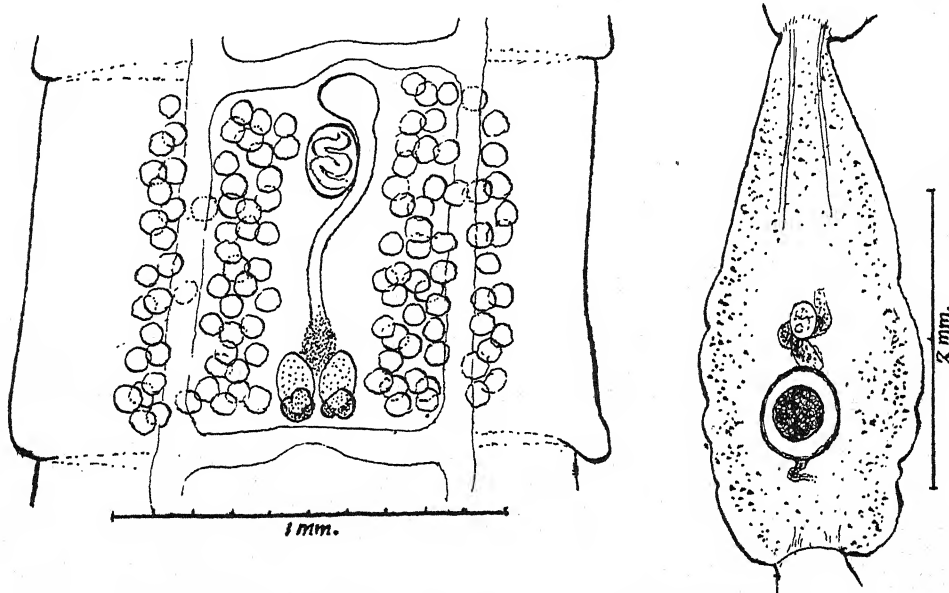


FIG. 1. Mature and gravid proglottids of *Mesocestoides kirbyi*, n. sp.

elongate, 120 to 170  $\mu$  long and 65 to 85  $\mu$  wide, almost contiguous. Vitelline glands ventral and slightly posterior to ovaries, 50 to 70  $\mu$  in diameter. Uterus curves around cirrus pouch, ending in club-shaped enlargement anterior to it. Egg sac forms as in *M. latus*, by thickening of walls of posterior part of uterus, differentiating into carrot-shaped organ with thick fibrous walls, posterior portion of which forms the capsule. Capsule 450 to 560  $\mu$  in diameter; egg mass 280 to 320  $\mu$  in diameter.

Host: *Canis latrans*.

Location: Intestine.

Locality: Hastings Natural History Reservation, California.

Type: U. S. Nat. Mus. Helm. Coll. No. 36898.

This species differs from all previously described forms in the large number of the testes, and from all but *M. lineatus* in the large size of the cirrus sac. It differs also from all except *M. manteri* in the bell-shape of the gravid proglottids. Except for the large number of testes this species has a strong resemblance to *M. lineatus*.—ASA C. CHANDLER, *Rice Institute, Houston, Texas*.

### TWO ERRONEOUS RECORDS IN AMERICAN LITERATURE OF THE CAUSATIVE AGENTS OF MYIASIS

Because of our relatively scant knowledge of the immature stages of Diptera, determinations of larvae are often erroneous, and some misidentifications are perpetuated through repeated publication. In the case of myiasis-producing species, another source of error is failure to interpret the clinical data correctly, infestations of the vaginal and anal regions, or contamination

of stools, being often misinterpreted as gastrointestinal myiasis. However, when records that are evidently based on accurate observation are accompanied by reasonably good photographs or drawings, they leave something tangible for future workers to consider.

Blankmeyer (1907, J. Am. Med. Assn. 48: 1505) reported a severe case of intestinal myiasis caused by *Fannia canicularis* (L.) (reported as *Anthomyia canicularis*). Later (1914, J. Am. Med. Assn. 63: 321), for some unstated reason, he decided that his previous determination was wrong and that the larvae in question were *Anthomyia pluvialis* (L.). But for a European record, which needs reexamination, this is the only record of this latter species as a producer of myiasis in man that the writer has been able to find. The drawing in Blankmeyer's first paper indicates clearly, however, that he was dealing with a species of *Fannia*, possibly *F. scalaris* (F.); it was not *F. canicularis* and most certainly not *Anthomyia pluvialis*, which is of an entirely different type.

Felt (1928, N. Y. State Mus. Bul. 274: 145-176) recorded the removal of several larvae of *Wohlfahrtia vigil* (Walker) from a cyst in the eye of a man in New York State. The record was unusual, since *W. vigil* is known to attack only subcutaneous tissues in very young animals, including the human species. It had not previously been reported in ocular myiasis, or in adults. The photograph accompanying Felt's report, however, is clearly that of the larva of *Oestrus ovis*, which has frequently been recorded as producing conjunctivitis in man.

Both these erroneous records have been cited by subsequent workers and textbooks of medical entomology.—MAURICE T. JAMES, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture.

#### PHLEBOTOMUS LIMAI FONSECA IN THE UNITED STATES (DIPTERA: PSYCHODIDAE)

In the United States there are five known species of *Phlebotomus*: *P. vexator* Coquillett, which is widespread in the southern states, having been taken from Maryland to California; *P. diabolicus* Hall, from Texas; *P. texanus* Dampf, from Texas; *P. stewarti*, a species which is being described from California by Mangabeira and Galindo; and the fifth is a species which I first collected from a hollow tree at Annie Pond, near Florence, Alabama. Dampf (1938, An. Fac. Nac. Biol. 1: 119) mentions another species from Florida, but we do not as yet know whether it is one of those already described or a new one.

The specimens from northern Alabama are so close to *P. limai* Fonseca that, in spite of a few differences in the morphology, I do not believe they can be considered to represent a new species. The female of *P. limai* has been redescribed by Coutinho (1940, Rev. Mus. Paul. 1: 333), and the male by Barretto and Coutinho (1940, An. Fac. Med. Univ. S. Paulo 16: 127). Table 1 gives a comparison of the measurements recorded for *limai* by these authors with measurements of 4 males and 5 females from Alabama. The palps are practically identical. In the

TABLE 1.—Comparison of *P. limai* from Brazil and Alabama  
(Measurements in microns)

	Females		Males	
	Brazil	Alabama	Brazil	Alabama
Palp				
I .....		47-52	24-32	31-36
II .....	170-189	161-177	121-135	125-135
III .....	148-183	161-177	108-121	125-135
IV .....	72-86	77-88	54-67	68-77
V .....	175-217	203-229	162-216	198-244
Wing				
$\alpha$ .....	629-757	560-620	483-583	460-530
$\beta$ .....	221-314	300-360	232-244	270-340
$\gamma$ .....	221-349	210-270	151-209	200-230
$\delta$ .....	189-279	140-250	116-233	120-170
$\alpha/\beta$ .....	2.0-3.1	1.7-2.0	2.0-2.5	1.4-2.0
Spermatheca				
Length .....	70	60-80	.....	.....

wing one can find the only real difference between the two forms; in the Alabama specimens  $\alpha$  is shorter and  $\beta$  is longer, so that  $\alpha/\beta$  is somewhat larger in the flies from Brazil. The male terminalia are identical. The only difference in the spermatheca seems to be a longitudinal seam that may occur in some of the North American specimens. Mr. R. M. Damasceno, who examined several specimens which I had given to Dr. P. C. A. Antunes, sent me several drawings which emphasize the differences mentioned above, but a study of the larger amount of material I have

indicates that these differences are not clear-cut. Dr. Ayroza Galvao agrees with me that the two are closely enough related to be considered as belonging to the same species.

The presence of *P. limai* in the United States presents an interesting problem in distribution. So far it has been reported only from several localities in the State of Sao Paulo, Brazil (Fonseca, 1935, 12th Cong. Int. Zool.: 1497; Coutinho, 1940; Barretto and Coutinho, 1940) where it does not seem to be particularly abundant (Galvao and Coutinho, 1940, Rev. Ent. 2: 427). In addition to the flies captured in the hollow tree at Annie Pond in Alabama, I have identified specimens in the collection of the U. S. National Museum from Clinton, Mississippi, and Rockingham, North Carolina, as well as specimens in the Chicago Natural History Museum from Rockingham, North Carolina, and Selma, Alabama. Some of the females had been taken while biting. It would seem that the species has a wide distribution in the southern United States and one might expect to find it in Central America and northern South America, or perhaps in Florida and southward through the West Indies. I have tentatively identified as *limai* several specimens that Dr. Pablo Anduze sent to me from Venezuela.—L. E. ROZEBOOM, Department of Parasitology, Johns Hopkins School of Hygiene and Public Health, and the Health and Safety Department, Tennessee Valley Authority.

#### BARROELLA N. NOM. FOR KIRBYELLA ZELIFF, 1930, HOMONYM

Zeliff (1930, Am. J. Hyg. 11: 740-742) described a flagellate from the termite, *Kaloterмес* (*Calcariterмес*) *brevicollis*, and proposed a new genus, *Kirbyella*, to contain it. The name *Kirbyella* had been given previously (Kirkaldy, 1906, Entomologist 39: 248) to a genus of insects and was not available. Accordingly, a new genus *Barroella* is erected to contain the flagellate species and the new combination *Barroella zeteki* is formed to replace *Kirbyella zeteki*.—C. COURSON ZELIFF, Pennsylvania State College.

#### CANCELLATION OF PROGRAM

The program of the American Society of Parasitologists, scheduled for Cleveland, Ohio, September 11 and 12, 1944, was cancelled except for the annual business meeting of the Society and the Symposium which was arranged jointly with the American Society of Zoologists, the American Society of Tropical Medicine and Section N (Medical Sciences) of the American Association for the Advancement of Science.

Titles and abstracts of papers submitted for the Cleveland meeting, together with a list of officers and new members, are published in the Supplement to the August number of the Journal of Parasitology.



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## STUDIES ON THE LIFE HISTORY OF *EURYTREMA PROCYONIS* DENTON, 1942<sup>1</sup>

J. FRED DENTON<sup>2</sup>

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### INTRODUCTION

Knowledge of the life cycles of trematodes of the subfamily DICROCOELIINAE Looss, 1899, exists for a single species, *Dicrocoelium dendriticum* (Rudolphi, 1819). The cercaria, *Cercaria vitrina* von Linstow, 1887, of this species has been known for years, but only recently has its complete development in the molluscan hosts been studied and its relationship to the adult proven. Vogel (1929) described previously unreported anatomical details of *C. vitrina* and concluded, on the basis of morphological similarity, that it was the cercaria of *D. dendriticum*. Cameron (1931) obtained cercariae which he identified as *C. vitrina* from snails, *Helicella itala* (= *H. ericetorum* Geyer, 1927), to which he had fed *D. dendriticum* eggs. Mattes (1933, 1936) observed the complete development of *C. vitrina* in snails, *H. ericetorum*, *H. candidula*, and *Zebrina detrita*, which he infected by feeding eggs. Finally, with the observations of Neuhaus (1936, 1938) of the encystment of the cercariae and the method of infection of the definitive hosts, the life cycle of *D. dendriticum* was completed.

Hoping to acquire knowledge of the life histories of additional species of the subfamily DICROCOELIINAE and possibly to determine the taxonomic value of larval characters for this group, a number of attempts were made between 1938 and 1941 to experimentally determine the life cycles of the common dicrocoeliids occurring in hosts in the vicinity of Houston, Texas. The present account, the first of a series, records results of the experiments on the life cycle of *Eurytrema procyonis* Denton, 1942.

### MATERIALS AND METHODS

From knowledge of the life cycle of *Dicrocoelium dendriticum*, it seemed likely that the molluscan host of *Eurytrema procyonis* would be a land snail. So a survey was made to determine the terrestrial snails occurring in Southeastern Texas. Living specimens of seven of the more common species were brought to the laboratory and breeding cultures were set up according to the methods described by Krull (1937). Excellent results were obtained in breeding all seven species. However, *Bulinulus alternatus mariae* grew too slowly in the laboratory for our purposes, so specimens for use in experiments were collected from a heavily populated area

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<sup>1</sup> A contribution from the Department of Biology, The Rice Institute, Houston, Texas.

<sup>2</sup> The author wishes to express his appreciation to Professor Asa C. Chandler under whose direction this investigation was done, and to Dr. Paul Bartsch of the U. S. National Museum for identification of the snail hosts.

where the snails were free of trematode infections as determined by examination of over 1,000 individuals.

Eggs used in the feeding experiments were obtained from the uteri of mature worms. After incubating the worms in tap water at 38–39° C for 48 hours, the embryonated eggs were teased out in a drop of water on a slide and examined to make certain that the miracidia were alive. A little cornmeal was then added and the eggs were fed to groups of 10 to 25 snails. To prevent the infection from being too heavy and killing the snails, an average of only 10 eggs per snail was fed. After 12 to 18 hours, droppings from the snails were examined for evidence of hatching. The snails were then placed in permanent cultures and kept for three months before being determined negative for infection. During the course of all experiments, the snails were given abundant food and the temperature of the laboratory remained at 18 to 25° C.

Sporocysts and cercariae were studied alive unstained, or stained *intra vitam* with neutral red and Nile blue sulphate. Measurements and sketches given in connection with this paper, unless otherwise stated, were made from fresh material killed with gentle heat. For permanent mounts, sporocysts and cercariae were fixed in hot Gilson's or Bouin's fixatives and stained with carmalum. Detail studies were made with the aid of either a high dry or an oil immersion lens.

#### BIOLOGY AND LIFE HISTORY

The adult *Eurytrema procyonis* is a common parasite in the interlobular ducts of the pancreas of raccoons, *Procyon lotor*, in Southeastern Texas. Six of ten animals examined from that locality harbored from 12 to more than 1,000 specimens of this trematode. The heavily infected raccoons showed no discernible effects from their infections.

Attempts to induce adult worms to discharge their mature eggs in physiological saline or tap water were unsuccessful. Also, many attempts to hatch eggs outside of the digestive tract of snails failed. Such attempts included incubation in tap water and 0.5 per cent saline at room temperature for various lengths of time, incubation in various dilutions of pepsin, and incubations in fluid from the stomach and intestine of snails.

Eggs of this worm were fed to *Polygyra texasiana* (Moricand), *Mesodon thyroidus* (Say), *Praticollega berlandieriana* (Moricand), *Angiuspira alternata* (Say), *Bulimulus alternatus mariae* (Albers), and *Deroceras agrestis* (Linnaeus). The eggs hatched in all six species of snails but development proceeded only in *Mesodon thyroidus*. Attempts to study living miracidia from the intestines of these snails were unsuccessful. The partly digested cornmeal produced a milky medium in which it was very difficult to observe the hatched miracidia. As well as could be determined all mature eggs had hatched after two hours, the hatching occurring in the upper small intestine of the host. The miracidia penetrated the intestinal wall in this region and passed to the middle digestive gland to continue their development.

Of 10 adult *M. thyroidus* to which eggs of *E. procyonis* were fed, seven became heavily infected, two failed to become infected, and one examined after 50 days may or may not have been infected.

It is probable that the developing mother sporocysts of *E. procyonis* can be detected with the dissecting microscope by the 50th day of infection. However, since *M. thyroidus* was not known to be a suitable host at that time, only one snail was sacrificed with negative results. A second specimen, examined on the 70th day of infection, contained several mother sporocysts which were just maturing. These sporocysts were located in the haemocoelic space, deep between the lobes of the middle digestive gland and contained daughter sporocysts in all stages of development from undifferentiated germ balls to recognizable sporocysts. Approximately 100 daughter sporocysts which had already ruptured from the parent forms were present in the digestive gland and along the adjacent intestine. In a snail autopsied on the 95th day of infection, the mother sporocysts were still intact and contained daughter sporocysts in all stages of development. In addition, over 2,000 daughter sporocysts in various stages of development were distributed throughout the digestive gland and other tissues of the snail.

Although no mother sporocysts were observed in snails examined on later dates, the evidence indicates that mother sporocysts produce daughter sporocysts continuously over a period of a month and probably longer, rather than a single generation of daughters which mature and rupture simultaneously from the parent.

The daughter sporocysts, after escaping from the parent, gradually migrate toward the respiratory portion of the mantle and the exposed parts of the snail's body. In a snail autopsied on the 95th day, the majority of these sporocysts were still concentrated in the digestive gland, although some of the largest and most developed ones had reached the wall of the mantle cavity where they were found in the branches of the pulmonary vein. Other daughter sporocysts were found in clusters in the collar and in the softer dorsal tissues of the body. In another snail examined on the 115th day, the branches of the pulmonary vein were greatly distended with daughter sporocysts. The collar and adjoining tissues were packed with them, and the foot, even the toughest part of it, contained many.

Some of the daughter sporocysts matured and were expelled on the 141st day of infection. From then until the experiment was terminated on the 177th day, the snails continued to expel masses of mature sporocysts daily. Observation of the snails revealed that the mature sporocysts rupture into the mantle cavity where they collect in groups of from two to three to as many as 125. The surfaces of the sporocysts are sticky, causing them to clump together. These clumps then become covered with mucus before being expelled from the respiratory pore of the host. As the snails crawl about, the expelled sporocysts are deposited on plants and other objects. In the humid atmosphere of the culture, sporocysts were expelled any time of day or night. Whether or not specific environmental factors affect the expulsion of sporocysts by snails was not determined.

When sporocysts are first expelled by snails, they are glistening white in appearance. After a short time the sporocyst wall dehydrates and shrivels, turning a light yellowish-brown color. This dead wall does not disintegrate but persists intact as a protective covering around the endocyst which encloses the cercariae. Cercariae in such sporocysts, deposited in a humid atmosphere or on the leaves of transpiring plants, remain alive for two or three days before disintegrating. In sporocysts placed in the refrigerator in 0.5 per cent saline, some cercariae were alive after five days.



At no time were cercariae found outside of the sporocyst in which they developed. Within the fluid which fills the body cavity of expelled sporocysts, the cercariae slowly extend and contract. Mechanical disturbance or immersion of the sporocyst in 0.5 per cent saline causes the cercariae to become more active. When the sporocyst wall and endocyst are ruptured, the freed cercariae crawl over the bottom of the container in geometrid fashion. The surface of the tail is sticky and appears to serve as an adhesive organ. After a few minutes of activity the cercariae become exhausted and lie motionless in the dish. They die in 6-8 hours.

At this point the question arose as to whether or not a second intermediate host is involved in the life cycle of this worm. Examination of two uninfected adult *M. thyroidus* which had been exposed for 25 days with the individuals expelling sporocysts daily, failed to reveal either free or encysted cercariae. Three half-grown *M. thyroidus* exposed for 14 days with snails expelling sporocysts also failed to show any cercariae. It was evident that *M. thyroidus* does not serve as a second intermediate host for this worm and it is unlikely that any other snail does. Whether or not some other animal serves in this capacity is still undetermined.

The fact that cercariae leave the molluscan host imprisoned in and protected by a tough endocyst suggested that infection of the definitive host may occur directly from eating snails containing mature sporocysts. Unfortunately, circumstances beyond our control abruptly terminated the experiments before this hypothesis could be tested. However, it was definitely determined (unpublished data) that in a *Eurytrema* from birds, direct infection of the definitive host does not occur.

#### DESCRIPTION OF DEVELOPMENTAL STAGES

*The egg and miracidium.*—The embryonated egg of *E. procyonis* (Fig. 1) is oval in outline, lemon-yellow in color, operculated, and measures 45-53  $\mu$  long by 29-36  $\mu$  wide. The lightly bile-stained shell is fairly thick but transparent. Usually many refractile droplets, probably excretory granules or disintegrating yolk, are present in the space between the shell and miracidium, particularly at the anterior end. The fully developed larva, orientated toward the operculum, exhibits practically no movement. Its body is pear-shaped and covered with cilia, most numerous and longest at the anterior end. Protruding slightly from the anterior end and extending posteriorly about one-third of the body length is a slender stylet. Within the anterior half of the body, the vague outline of the primitive gut and penetration glands can be detected. Two large, oval, oppositely placed vesicles, containing highly refractile granules, occupy most of the posterior half of the body. Between the gut and the refractile bodies several large cells, believed to be germ cells, are discernible. No epidermal plates or flame cells could be detected in either unhatched miracidia or miracidia forced from eggs by pressure.

*The mother sporocyst.*—The mother sporocyst as observed on the 70th day of infection is a pearly white, extensively lobed mass. Apparently, its shape is determined somewhat by the location in which it develops. It is of a very delicate texture and firmly attached to the tough connective tissue of the host, making separation very difficult. Externally it is covered by a thin delicate membrane composed of a single layer of flattened epithelial cells. Whether or not a thin cuticle is present was not determined. The mass of the body or matrix is composed of irregularly shaped cells with small round nuclei. The numerous developing daugh-

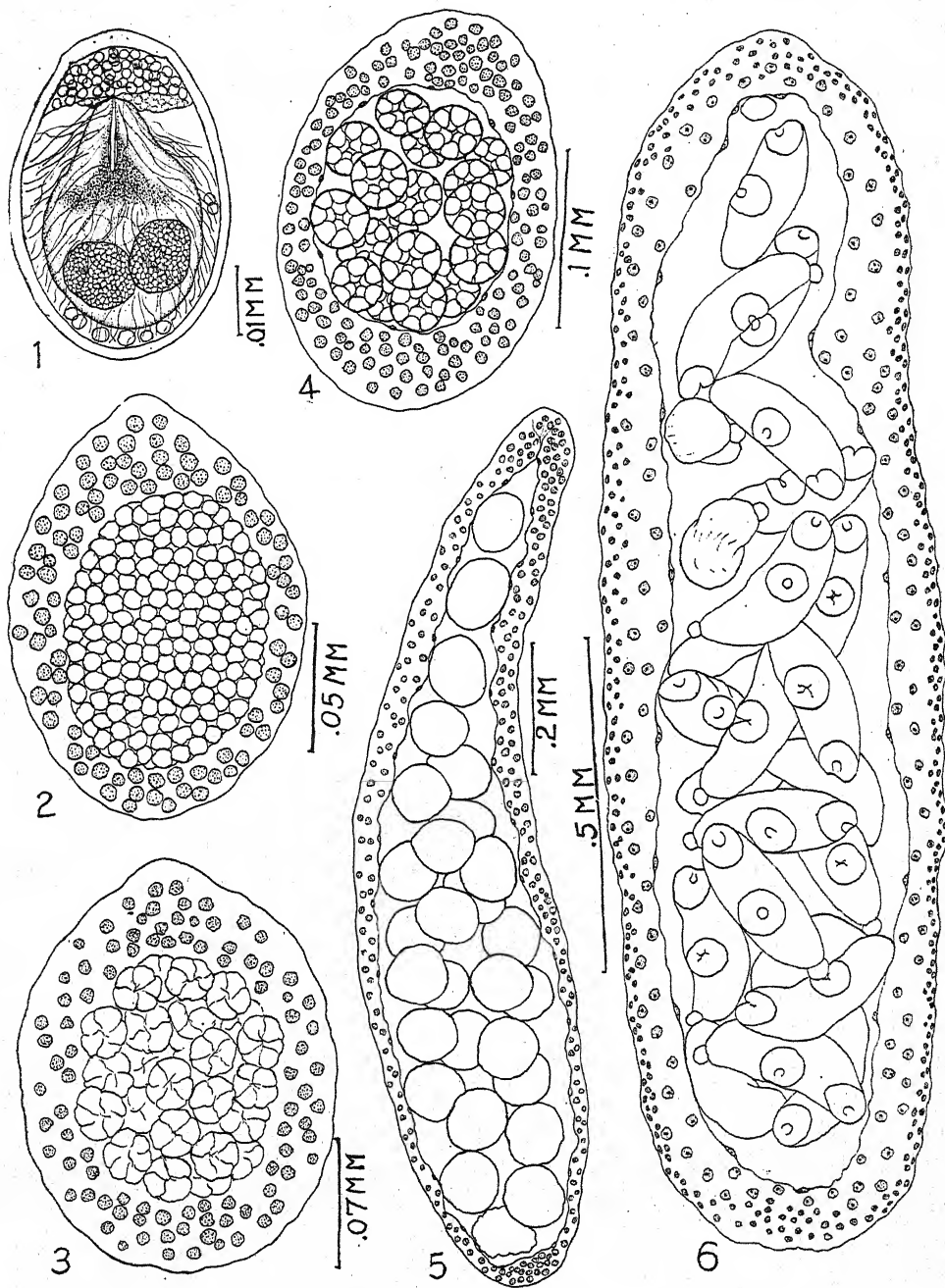


FIG. 1. Egg with mature miracidium, showing stylet and large refractive bodies.

FIG. 2. Immature daughter sporocyst recently ruptured from mother sporocyst, showing outer somatic portion and central mass of germ cells.

FIG. 3. Daughter sporocyst showing segmentation of the germ cell mass into cercarial "germ balls."

FIG. 4. Daughter sporocyst after formation of cercarial embryos and appearance of epithelial lining of body cavity.

FIG. 5. Daughter sporocyst on the 95th day of infection.

FIG. 6. Mature daughter sporocyst containing fully formed cercariae.

ter sporocysts are scattered through the matrix in cavities which lack definite cellular walls. The daughter sporocysts, which are most numerous near the periphery of the lobes, are in all stages of development from small germ balls to forms ready to leave the parent. Since daughter sporocysts were found free in the haemocoelic space at this time, the mother sporocyst was assumed to be mature.

Mother sporocysts observed in a snail examined on the 95th day of infection did not differ from those studied on an earlier date. They also contained daughter sporocysts in all stages of development. No mother sporocysts were found in snails autopsied at later dates.

*The daughter sporocyst.*—The youngest daughter sporocyst embryo contained within the matrix of a mother was in the "germ ball" stage. This embryo measured  $18\ \mu$  in diameter and consisted of four rounded germ cells enclosed by about 16 slightly smaller cuboidal cells. Various other embryos, in more developed stages, contained within the mothers, show a gradual increase in the number of germ cells with a corresponding increase in and flattening of the outer cells to form a membrane or wall one cell layer in thickness. An embryo which measured  $35\ \mu$  in diameter contained a central mass of approximately 16 germ cells. Still further developed daughter sporocysts show a gradual increase in the size of the germ cell mass with a concurrent thickening of the wall so that it becomes two or three cell layers thick.

The daughter sporocyst (Fig. 2), when it first ruptures from the parent form, averages 0.273 mm long by 0.150 mm wide. It is an oval, colorless, delicate, transparent form capable of slight movement. It is covered by a thin cuticle  $1\ \mu$  in thickness. The body is differentiated into an outer somatic portion consisting of several layers of irregularly shaped cells, and a central germ mass consisting of tightly packed, rounded germ cells. Although there is no definite membrane separating the somatic portion from the germ mass, the line of demarcation is very distinct in stained specimens. No flame cells or elements of the excretory system could be detected.

In somewhat older forms (Fig. 3) the somatic portion is unchanged but the germ mass is beginning to divide into a number of cercarial germ balls of equal size. In still further developed sporocysts (Fig. 4) the soma is completely separate and the body cavity resulting from this separation is lined by a definite membrane consisting of a single layer of flattened epithelial cells. The division of the germ mass into round cercarial germ balls is complete. Each cercarial embryo is covered by a thin membrane.

By the 95th day of infection these sporocysts (Fig. 5) had increased greatly in size, the largest measuring 1.42 mm long and 0.40 mm wide. The cuticle measured  $1.5\ \mu$  in thickness, while the entire wall, composed of three or four layers of cells, averaged  $25\ \mu$  in thickness except at the ends where it was much thicker. Within the relatively transparent walls of the living sporocyst many elements of a paired excretory system are now visible. The two excretory pores are located in opposite walls near the middle of the sporocyst. Just interior to each excretory pore is a small sinus from which arise four main collecting tubules. One main tubule runs anteriorly to supply the anterior end, another runs posteriorly to supply the opposite end, while the other two run laterally in opposite directions to supply the sides. Numerous flame cells are visible scattered throughout the wall. Within the body

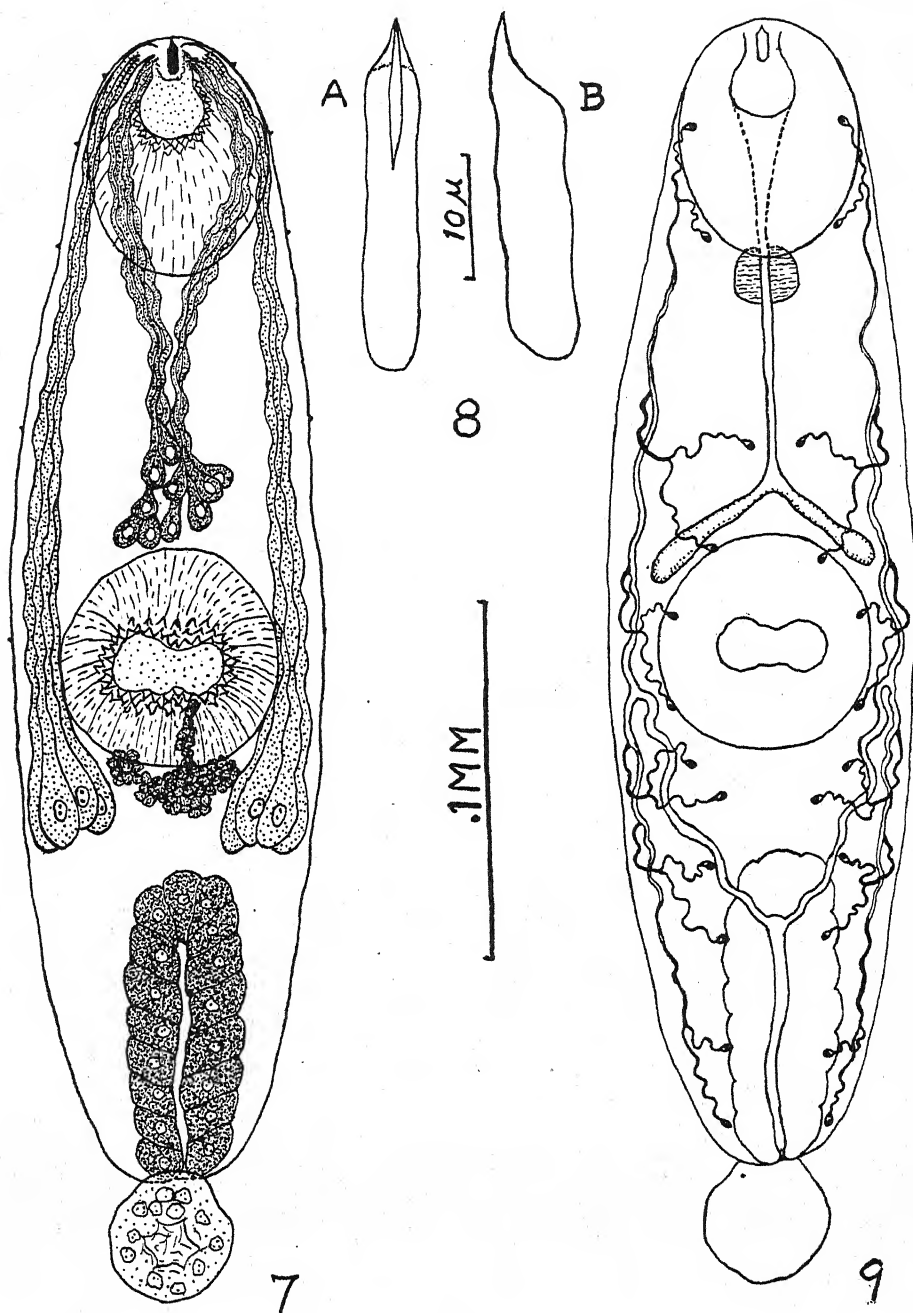


FIG. 7. Ventral view of cercaria showing morphological details.

FIG. 8. Stylet of cercaria. A—dorso-ventral view, B—lateral view.

FIG. 9. Excretory system of the cercaria. Freehand sketch on camera lucida outline.



cavity of the sporocyst the 20 to 40 developing cercarial embryos appear as ovoid bodies, all of which are approximately the same size and average 0.105 mm long by 0.080 mm wide. So far as could be determined, no increase in the number of developing cercariae had occurred since the initial division of the germ mass.

Mature daughter sporocysts (Fig. 6) when freshly expelled by the snails are pearly white in appearance. They are stout tubular structures with rounded ends, measuring 1.62–1.97 mm in length by 0.46–0.53 mm in diameter. The wall, which is covered by a cuticle, measures 0.045–0.105 mm in thickness. It consists of several layers of flattened cells with small nuclei and two or three layers of irregularly shaped cells with large rounded nuclei. The body cavity is lined by a membrane composed of a single layer of large flattened epithelial cells. Between the lining of the body cavity and the remainder of the sporocyst wall there is a tough noncellular layer, suggesting a cyst wall, approximately 1  $\mu$  in thickness. The cercariae are thus enclosed in a cyst-like structure lined on the inside by the layer of living epithelial cells and on the outside by the noncellular membrane. The name endocyst is proposed for this structure. There is no birth pore or canal penetrating the wall of the sporocyst. The body cavity is filled with a viscid fluid which was shown by *intra vitam* stains to be material from the large posterior glands of the cercariae. The 20 to 40 cercariae, all of which are mature, are suspended in this fluid.

*The cercaria.*—The cercaria (Fig. 7) is small, muscular, elongate oval in shape, and flattened dorso-ventrally. It measures 0.348–0.370 mm long by 0.095–0.105 mm wide in the region of the acetabulum. The thin cuticle, measuring 1–1.5  $\mu$  in thickness, is aspinose and smooth. In living specimens several pairs of sensory papillae are visible along the lateral margins of the anterior half of the body. The tail, which contains the nuclei of from 16 to 20 cells, is a short, rounded structure, measuring 28–38  $\mu$  diameter.

The muscular oral sucker is directed anteriorly but possesses a subterminal opening. It is oval in shape, measuring 0.059–0.063 mm long by 0.053–0.062 mm wide. The acetabulum, which is situated near the equator of the body, measures 0.064–0.071 mm in diameter. The exposed surfaces of both suckers are covered with very small scales or spines. Prepharynx absent; the pharynx is globular, muscular, and measures 20–21  $\mu$  long by 22–27  $\mu$  wide. The long slender esophagus bifurcates into two short ceca just anterior to the acetabulum.

The very small stylet (Fig. 8) measuring 26–30  $\mu$  in length, 5–7  $\mu$  in width, and 6–8  $\mu$  in depth, lies within a small pocket in the antero-dorsal wall of the oral sucker. This pocket communicates with the outside by a small opening in the anterior wall of the sucker.

On either side of the acetabulum and extending slightly posterior to it is a group of four large penetration glands. The ducts from these glands pass anteriorly by a slightly undulating course to the lateral walls of the oral sucker through which they open into the stylet pocket. The ducts on either side apparently fuse together and open into the stylet pocket through a common pore. Since these glands are detected with the greatest difficulty, it is likely that there are other dorsal to the acetabulum. Five pairs of smaller penetration glands occupy the midregion of the body between the oral sucker and acetabulum. The ducts from these glands, five on each side of the midline, pass anteriorly to open into the stylet pocket just medial to the large gland ducts.

The excretory pore is terminal and dorsal to the subterminally attached tail. The simple, tubular, I-shaped vesicle (Figs. 7, 9) which is composed of a layer of large dense cells, extends anteriorly about two-thirds the distance to the acetabulum. At its anterior end it receives a common collecting tubule from each side of the body. The two common collecting tubules pass anteriorly and laterally to about the equator of the acetabulum where each branches into an anterior and posterior main collecting tubule. The anterior main collecting tubules on each side pass forward to give rise to three accessory tubules, each of which branches into two capillaries. Similarly, the other two main collecting tubules pass posteriorly, giving rise to three accessory tubules, each of which branches into two capillaries. Each capillary tubule terminates in a single flame cell establishing a flame cell pattern of the type  $2 [(2+2+2) + (2+2+2)]$  for this cercaria.

In stained specimens the genital primordium appears as a deeply-stained mass of small cells lying in the midline just posterior to the acetabulum. From the main portion of the primordium a chain of cells extends anteriorly dorsal to the acetabulum.

#### DISCUSSION

The development of the larval stages of *E. procyonis*, as determined in experimentally infected snails, differs in several respects from that described for *Dicrocoelium dendriticum* by Mattes. Instead of producing a single generation of daughter sporocysts all of which mature simultaneously as reported for *D. dendriticum*, the mother sporocyst of *E. procyonis* produces daughter sporocysts continuously over a period of a month or more. The mature daughter sporocyst of *D. dendriticum* possesses a wall one cell layer thick except at the ends, and is provided with a birth canal. Cercariae develop and escape continuously from these sporocysts over a period of several months. In contrast, the mature daughter sporocyst of *E. procyonis* possesses a wall several cell layers thick, the inner of which is an epithelial membrane lining the body cavity. It has no birth canal and the cercariae which develop simultaneously from germ balls produced by the division of the germ mass in the immature form, remain confined in the sporocyst.

There is also a difference in the manner in which the cercariae of the two species leave their molluscan hosts and are protected after their departure. According to Neuhaus, the cercariae of *D. dendriticum* migrate out of the daughter sporocysts through the birth canal and actively penetrate into the mantle cavity of the snail. Here they collect in groups of 200 to 400 and form a common spherical cyst from the secretion of their large glands. These group cysts are forced to the respiratory opening by the breathing movements of the snail. Here from five to fifteen of these cysts adhere together in a large grape-like mass which becomes covered with mucus from the snail. As the snails crawl about, these masses are deposited on vegetation and other objects. In *E. procyonis*, where the cercariae are confined within the body cavities of the mature daughter sporocysts, the sporocysts themselves either migrate or rupture into the mantle cavity of the host where they collect in clumps. These masses of sporocysts, which become covered with mucus from the snail, are then expelled from the respiratory pore and are deposited on vegetation and other objects.

There is no difference in the fundamental morphology of the cercariae of *D. dendriticum* and *E. procyonis*. Other than the difference in the size and state of

degeneration of the tail, the two forms are strikingly similar in possessing a stylet, both large and small penetration glands, and a flame cell formula of 2  $[(2+2+2) + (2+2+2)]$ . This similarity suggests that cercarial characters, particularly the flame cell formula, when they are known for other species of the family, will be useful in eradicating the taxonomic confusion existing within the DICROCOELIIDAE.

## SUMMARY

Ova of *Eurytrema procyonis* Denton, 1942, when fed to the common garden snail, *Mesodon thyroideus* (Say), develop into mature mother sporocysts in 70 days. The mother sporocysts which develop in the haemocoelic space of the digestive gland are extensively lobed white masses. Within the matrix of the mother, daughter sporocysts are produced over a period of a month or more. The daughter sporocysts, after rupturing from the parent form, migrate to the respiratory portion of the mantle and surrounding tissues of the host where they mature in 141 days. Within the body cavity of the daughter sporocysts, a "fixed" number of cercariae are produced by the equal division into germ balls of the central mass of single germ cells present in them at an early stage of development. The daughter sporocysts, when mature, rupture into the mantle cavity of the host where they collect in clumps. These clumps of sporocysts are expelled from the respiratory pore by the movements of the snail and are deposited on vegetation and other objects. The styleted, almost tailless cercariae which have a flame cell formula of 2  $[(2+2+2) + (2+2+2)]$  remain confined within the body cavity of the sporocyst. Whether or not a second intermediate host is involved and the mode of infection of the definitive host was not determined.

The larval forms of *E. procyonis* are compared with those of *Dicrocoelium dendriticum* (Rudolphi, 1819), the only other species of the subfamily DICROCOELIINAE Looss, 1899, for which they are known. The mother sporocyst of *E. procyonis* is similar in morphology to that of *D. dendriticum* but differs in its manner of producing daughter sporocysts. The daughter sporocysts of the two species differ both morphologically and in the manner and number of cercariae produced. The cercariae of *E. procyonis* and *D. dendriticum*, which have the same flame cell formula, are similar in their fundamental morphology.

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IXODES OZARKUS N. SP. AND ORNITHODOROS AQUILAE N. SP.,  
WITH NOTES ON O. TALAJE AND O. KELLEYI (IXODOIDEA)\*

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Three lots of a new species of *Ixodes* have been received from Dr. W. J. Baerg, University of Arkansas, Fayetteville, Arkansas. The accession numbers are as follows:

20760, Dog, Huntsville, Arkansas, Feb. 20, 1944, 3 females, 2 males; 20854, Deer, Huntsville, Arkansas, Feb. 20, 1944, 3 females, 2 males; 21023, Dog, Huntsville, Arkansas, March 1944, 3 females.

Dr. Baerg informed me that some specimens of this tick had also been taken from raccoon and that the tick readily attacks man. He further states, "When attached it is difficult to remove, and the effects on man seem to persist much longer than the effects of the common dog tick. This species of tick has been very active, getting on hunting dogs in large numbers during the months of December and January, as well as in February." The tick is described and figured below.

*Ixodes ozarkus* n. sp.

(Figs. 1 and 2)

Female

**Body:** Length (unengorged) 2.46 mm; width 1.65 mm. Oval, widest at about the middle. Color of well-sclerotized parts dark brown (nearly black). Scutum extending about half the length. Postscutal area finely and faintly striated, and with long fine hairs similar to those on the scutum. Marginal grooves distinct anteriorly, faint posteriorly. No fully fed females available for description.

**Capitulum:** Length 0.84 mm; width of basis (just posterior to palpi) 0.54. Basis with lateral profile lines mildly curved and converging posteriorly. Posterior margin nearly straight and with the salient edge slightly depressed over the distinct, small, pointed cornua. Porose areas large, depressed and separated by about the length of the shorter axis of one. Surface smooth, shining, faintly shagreened, impunctate and without hairs. Palpi long with articles 2 a little longer than 3. Combined length of 2 and 3, 0.69 mm. Greatest width 0.22 mm. Outer profile lines nearly straight, inner edges curved. Surface smooth, shining and with a few faint punctations. Hairs few and moderate in size.

In ventral view, basis is mildly constricted at the middle. Posterior margin broadly rounded, salient. Transverse sutural line distinct. Auriculae distinct as lateral saliences. Surface smooth, shagreened, impunctate and with a very few short hairs at the sides. Palpi flattened on their inner faces. Article 1 with an indistinct ventral plate.

**Hypostome:** Long, with sides approaching parallel, apex rounded. Denticles 3/3 for about three-fourths the length, then 2/2 to the base. Lateral denticles large, pointed; medians progressively smaller to the middle, rounded. Length 0.60 mm.

**Legs:** Long, smooth, faintly shagreened. Tarsi I especially long. Haller's organ noticeably distant from the distal end. Length of tarsus I, 0.71 mm; metatarsus, 0.48 mm. Length of tarsus IV, 0.66 mm; metatarsus, 0.48 mm.

**Coxae:** Coxae II, III and IV mildly convex, I, more convex. Coxa I with a long pointed inner spur, which overlaps II in unfed and little fed specimens. External spur on I very short and connected with the internal spur by a continuous edge which limits the posterior side of the coxa. External spurs on II, III and IV about the same as that on I. Coxal hairs long or short and few in number.

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\* Contribution from Rocky Mountain Laboratory (Hamilton, Montana), Division of Infectious Diseases, National Institute of Health.



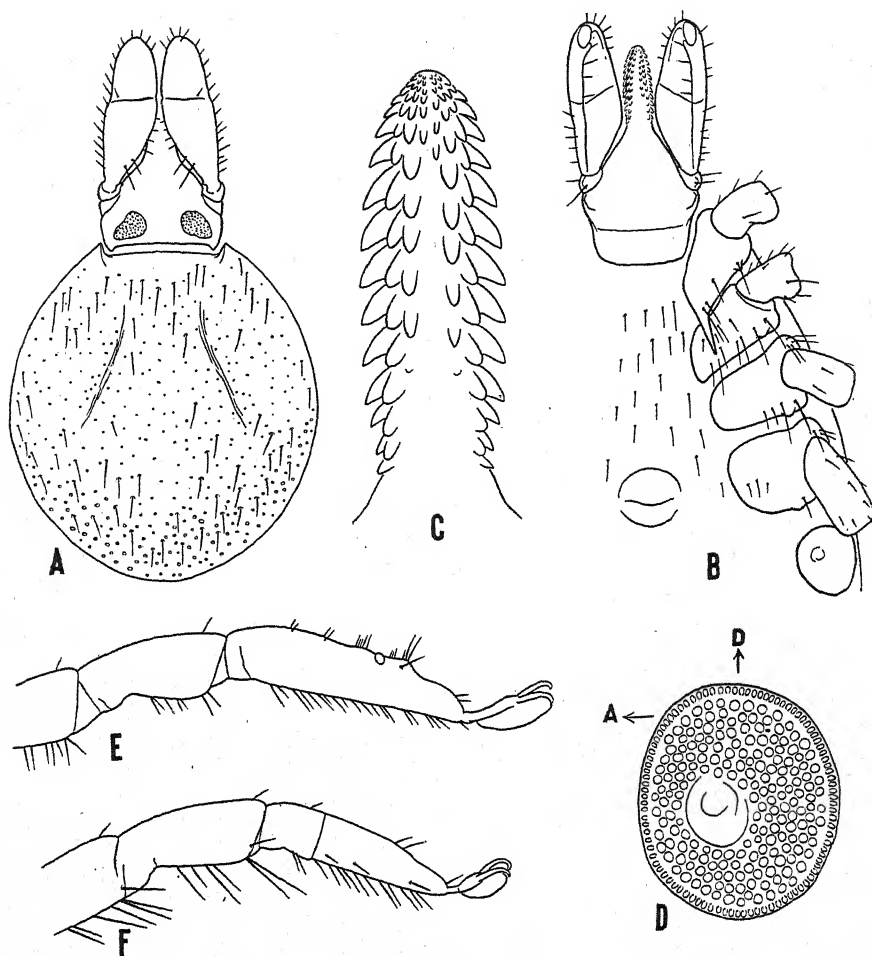


FIG. 1. *Ixodes ozarkus* n. sp., female. A. Capitulum and scutum, dorsal view. B. Capitulum and coxae, ventral view. C. Hypostome. D. Spiracular plate. E. Tarsus and metatarsus of leg I. F. Tarsus and metatarsus of leg IV.

*Spiracular plate*: Subcircular with the slightly longer axis transverse. Goblets moderate in size and number. Surface of plate nearly level. Length 0.39 mm, width 0.33 mm.

*Sexual aperture*: Situated between coxae IV.

#### Male

*Body*: Oval, widest at about the middle. Color as in the female. Length 2.40 mm, width 1.26 mm.

*Capitulum*: Dorsum of basis large, broad and flattened. Greatest width of basis 0.30 mm. Lateral profile lines nearly straight and converging posteriorly. Posterior margin straight or a little convex. Cornua absent. Lateral and posterior edges salient. Surface shagreened, punctate on the posterior half; and without hairs. Palpi short and broad. Articles 2 and 3 about equal in length, suture separating them depressed. Hairs for the most part short, but a few at the base are long.

In ventral view, basis is triangular with posterior margin a rounded salient point which is raised over the level of the "neck." Auriculae as small, rounded saliences. Palpi flattened on their inner faces. Article 1 visible as a ridge which is continuous with the posterior edge of 2 and 3 and has 2-3 hairs. Surface smooth, shagreened, impunctate and without hairs.

*Hypostome*: Short and broad and of the type found in *I. reduvius* (the genotype), *I. scapularis* and *I. pacificus*. Lateral denticles large and with the proximal pair especially large and

rounded; medians principally as transverse crenulations. Distal end of hypostome rounded or very faintly notched; basal portion free of denticles. Length about 0.26 mm.

**Scutum:** All peripheral areas about equally declivitous. Limits of pseudo-scutum not visible. Scapulae rounded. Lateral carinae absent. Cervical grooves faint, first convergent, then divergent; better seen in reflected light. Surface dulled by the distinct shagreening. Supine hairs numerous, long and fine, about evenly distributed; similar to those on the lateral folds. Puncta-

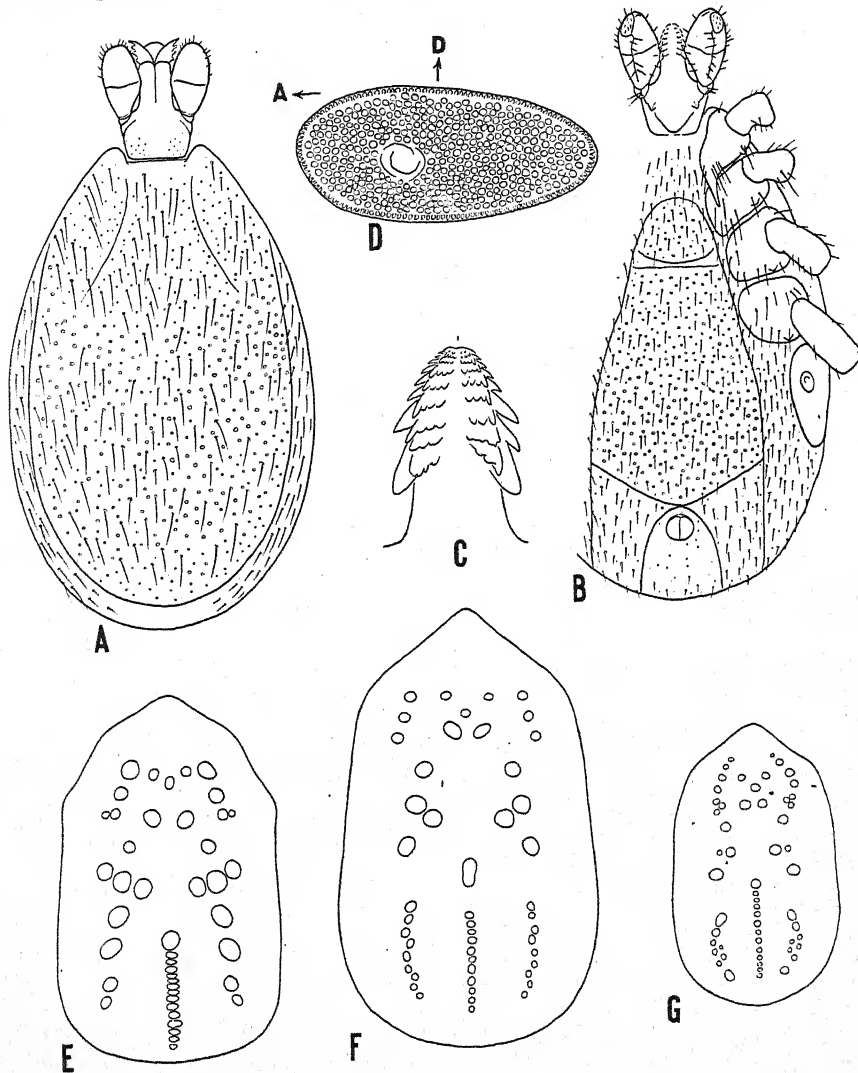


FIG. 2. *Ixodes ozarkus* n. sp., male. A. Capitulum and scutum, dorsal view. B. Capitulum, coxae, and plates, ventral view. C. Hypostome. D. Spiracular plate. E. *Ornithodoros talaje*, female, showing shape, relative size, and pattern of discs. F. *Ornithodoros kelleyi*, female, showing shape, relative size, and pattern of discs. G. *Ornithodoros aquilae* n. sp., female, showing shape, relative size, and pattern of discs.

tions numerous, distinct and of moderate size, those in the central and lateral areas usually larger than those on the ends.

**Ventral plates:** Median plate about three times as long as the anal. Anal plate much wider behind, adanals much wider in front. Median and adanal plates with numerous distinct punctations; anal plate with only fine punctations. Hairs numerous and like those on the scutum excepting that they are fewer and shorter on the anal plate and on the posterior part of the adanals.

**Legs and coxae:** Essentially as in the female.

*Spiracular plate*: Oval, large, with longer axis longitudinal. Surface following the contour of the body wall. Goblets very numerous. Length 0.48 mm, width 0.24 mm.

*Sexual aperture*: Between coxae III.

Nymphs and larvae unknown.

*Ixodes ozarkus* resembles *I. scapularis*, which occurs in the same area, and *I. affinis*, which occurs in Central America and South America. *I. ozarkus* might easily be confused with *I. affinis* but in the latter the female scutum is more nearly circular and has conspicuous, large, deep punctations near the posterior margin. The female hypostome of *I. affinis* has four large files of denticles on each side, while *I. ozarkus* has only three. *I. affinis* has a distinct ventral plate on palpal article 1, and lacks the small cornua found in *I. ozarkus*. The male of *I. affinis* has larger, more distinct punctations on the median plate and has large, distinct punctations on the scutum which are limited to the central area. The female of *I. scapularis* is distinguished from the female *I. ozarkus* by having fewer hairs on the scutum, shorter files 3 of the denticles on the hypostome, and shorter spurs on coxae 1. The males of *I. scapularis* are smaller than in *I. ozarkus*, have the hypostome relatively narrow and the hairs on the dorsum shorter and less noticeable.

*Holotype*: Female from 20760.

*Allotype*: Male from 20760.

*Paratypes*: Females and males from 20760, 20854, and 21023.

Holotype, allotype, and paratypes are deposited in the Rocky Mountain Laboratory. Other types have been placed in the following: U. S. National Museum, Washington, D. C., paratype female and male; Department of Entomology, University of Arkansas, Fayetteville, Arkansas, paratype female and male; Division of Entomology and Economic Zoology, University of Minnesota, University Farm, St. Paul, Minn., paratypes female and male; Museum of Comparative Zoology, Harvard University, Cambridge, Mass., paratype female; Department of Entomology, University of California, Berkeley, Calif., paratype female.

Beginning in May 1943, Dr. R. B. Williams, Director, Wyoming Public Health Board, Cheyenne, Wyoming, sent us immature *Ornithodoros* ticks taken as larvae from the region of the head of raptorial birds. Some had molted into the first nymphal stage when received. These ticks were assigned accession numbers as follows:

20247, *Falco mexicanus*, Chalk Bluffs, Weld Co., Colorado, May 31, 1944, 12 larvae; 20262, *Falco mexicanus*, Chalk Bluffs, Weld Co., Colorado, Elev. 5780. June 16, 1943, 9 larvae; 20263, *Buteo regalis*, Chalk Bluffs, Weld Co., Colorado, June 16, 1943, 17 larvae; 20264, *Falco mexicanus*, Chalk Bluffs, Weld Co., Colorado, June 16, 1943, 6 larvae; 20267, *Aquila chrysaetos canadensis*, Simpson Creek, Weld Co., Colorado, June 19, 1943, many larvae; 20269, *Falco mexicanus*, Pawnee Buttes, Weld Co., Colorado, June 20, 1943, many larvae; 20270, *Aquila canadensis*, Big Simpson Creek, Weld Co., Colorado, June 20, 1943, many larvae; 20275, *Aquila chrysaetos canadensis*, near Chugwater, Platte Co., Wyoming, June 26, 1943, 13 larvae; 20278, *Aquila chrysaetos canadensis*, Big Simpson Creek, Weld Co., Colorado, July 4, 1943, 27 nymphs; 20279, *Aquila chrysaetos canadensis*, Chalk Bluffs, Weld Co., Colorado, July 3, 1943, 18 nymphs; 20280, *Falco sparverius sparverius*, Big Simpson Creek, Weld Co., Colorado, July 3, 1943, 1 nymph; 20281, *Falco mexicanus*, Chalk Bluffs, Weld Co., Colorado, July 4, 1943, 11 nymphs.

The larvae were first fed on a hawk, and later on mice and guinea pigs. Attempts to feed them on a young chicken were not successful. Some of the larvae were reared to the adult stage and five females and four males were obtained.

From larval, nymphal, and especially from adult characters, the ticks were found to be a new species which is described and figured below.

*Ornithodoros aquilae* n. sp.

(Figs. 2 and 3)

Adult

**Body:** Oval with sides nearly parallel, and with excavations near legs I and IV variable (usually less pronounced than in either *O. kelleyi* or *O. talaje*). Anterior margin extended into a rounded deflexed point in front. Size of female: largest of five, 5.10 long by 2.49 wide; smallest of five 3.90 by 1.95 wide; average length and width 4.53 by 2.49. Size of male: largest of four, 3.90 long by 1.92 wide; smallest of four 3.48 long by 1.65 wide; average length and width 3.74 by 1.83.

**Mammillae:** On dorsal surface the mammillae are large, placed close together but not crowded; about equal in size in median and peripheral areas, distinctly larger in posterior than in anterior areas. The best defined individual mammillae show definite ridges on the sides which radiate from top to base; many have each an imperfect depression at the top which continues as a groove extending to near the base. On the venter, the mammillae in the peripheral areas are as large as those on the dorsum with those in the median areas becoming progressively smaller and less definite to the middle, excepting that those on the supracoxal folds are smallest and least definite.

**Discs:** On dorsal surface, large, conspicuous and with their immediate edges raised, those in the median area separated by about two to four staggered rows of mammillae. On ventral surface, discs are limited to the paired preanal and the median postanal grooves in which they are in lineal arrangement and present also in three groups caudad of the transverse postanal groove.

**Legs:** Small and of moderate length; surface micromammillated; hairs small. Length of female tarsus I, 0.6; metatarsus, 0.48. Length of female tarsus IV, 0.72; metatarsus, 0.6.

**Coxae:** Coxae I and II separated, all others contiguous. Surfaces partly micromammillated and partly with excrescences.

**Hood:** Not much in evidence, merging into the anterior extensions of the supracoxal folds, anterior end terminated by the deflexed, rounded anterior point of the body wall.

**Cheeks:** Large, suboval, and when apposed largely covering the palpi and hypostome. Both ends free. Surface very irregular.

**Capitulum:** Quadrangulate, much wider than long, surface micromammillated and with transverse wrinkles; hairs absent excepting 1 pair well back of hypostome and in addition to the usual posthypostomal hairs. Papal article 1 micromammillated.

**Hypostome:** Short and small, sides nearly parallel, mildly notched apically. Denticles with two short files of large denticles on each side, and with a third pair of very small denticles which are lacking in *O. talaje* and *O. kelleyi*. Length about 0.32 (female), 0.20 (male).

**Folds:** Coxal and supracoxal folds present, the latter extending anteriorly into the faint hood.

**Grooves:** Preanal, transverse postanal and median postanal grooves present. Dorso-ventral grooves absent.

**Sexual aperture:** At the level of the intervals between coxae I and II in the female; between coxae I in the male.

**Eyes:** Absent.

Nymph

As in most species of *Ornithodoros*, late stage nymphs of this species differ from the adults principally in size and absence of the sexual aperture.

Larva

Unfed larva suboval; basis capituli triangular in dorsal view. Palpi very long, slender and in life appressed against the hypostome. Average length of body including the capitulum 0.64.

**Hypostome:** Long and slender, but less slender than in *O. kelleyi*; denticles 3/3 for about the apical one-third, then 2/2 to the base; medians much less visible than the laterals even in specimens mounted in balsam. Length, apex to basal denticles, 0.64.

**Holotype:** Reared female from 20278.

**Allotype:** Reared male from 20278.

**Paratypes:** Adults reared from Nos. 20247, 20262, 20263, 20270, 20278.

Paratype larvae from 20270.



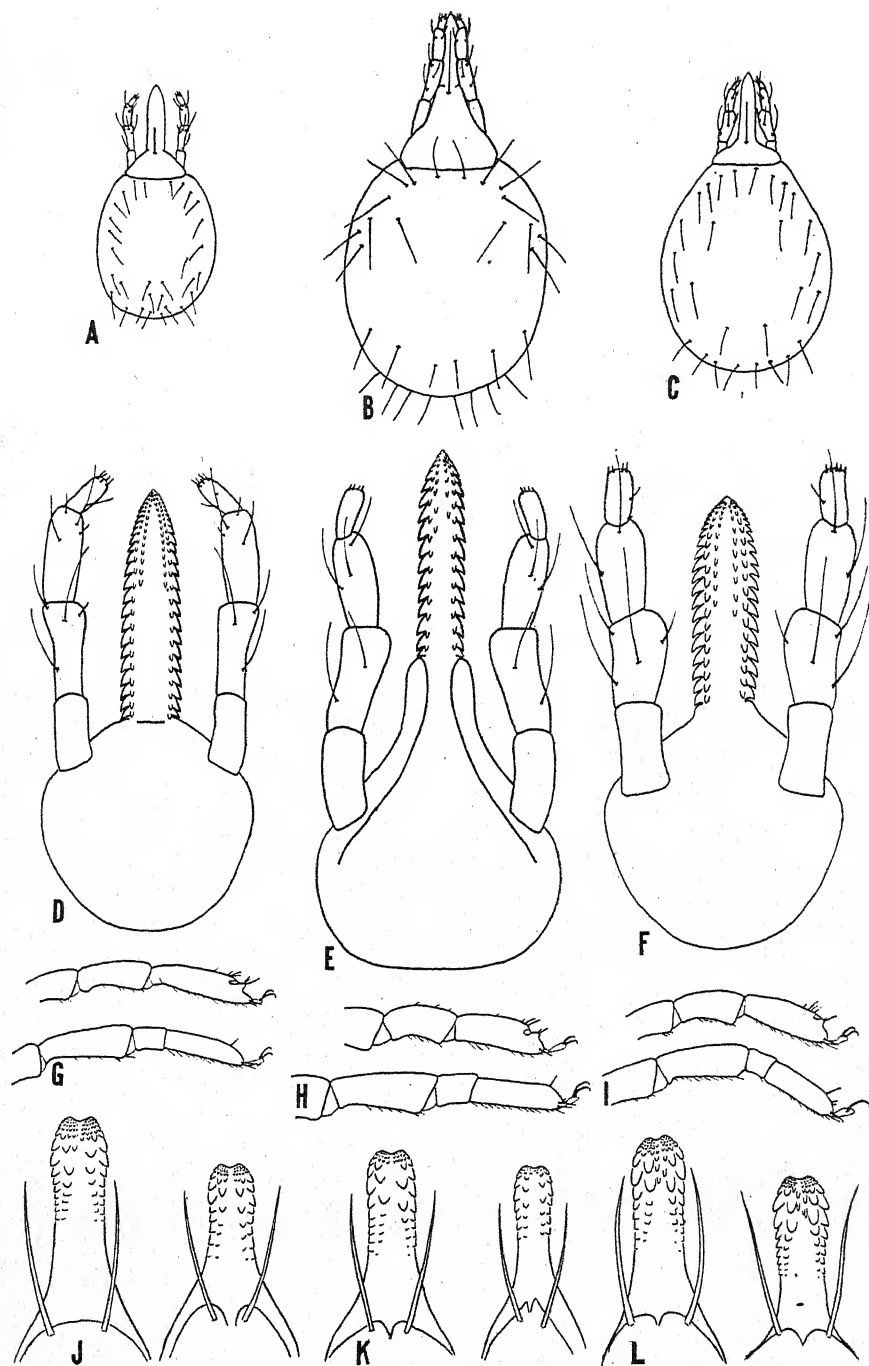


FIG. 3. Each part shown in relative size. A. *O. talaje* larva, capitulum and body, dorsal view. B. Same of *O. kelleyi*. C. Same of *O. aquilae*. D. *O. talaje* larva, capitulum, ventral view. E. Same of *O. kelleyi*. F. Same of *O. aquilae*. G. *O. talaje*, female, tarsus and metatarsus of legs I and IV. H. Same of *O. kelleyi*. I. Same of *O. aquilae*. J. *O. talaje*, hypostome of female (on left) and male. K. Same of *O. kelleyi*. L. Same of *O. aquilae*.

Holotype, female, allotype, male, and paratype larvae deposited in the collection of the Rocky Mountain Laboratory.

Paratypes male, female, and larvae deposited in the U. S. National Museum, Washington, D. C., the Museum of Comparative Zoology, Harvard University, Cambridge, Mass., and the Division of Entomology and Economic Zoology, University of Minnesota, University Farm, St. Paul, Minnesota.

*O. aquillae*, *O. talaje*, AND *O. kelleyi* COMPARED

*O. aquillae* and *O. kelleyi* Cooley and Kohls, 1941, are both closely related to *O. talaje* (Guérin-Ménéville, 1849) and could easily be confused with it. Species that have been incorrectly identified as *O. talaje* in the past and have been so referred to in the literature are: *O. hermsi*, *O. rudis*, *O. coprophilus* (now *Antricola coprophilus*), and *O. migonei*. This confusion resulted from the inadequacy of the early descriptions of *O. talaje*.

"*O. talaje*" in past years has been reported from Venezuela, Mexico, New Granada, Santiago de Chile, Colombia, Hawaii, Florida, Texas, and California, and in view of recent findings it becomes apparent that further studies may show that some of the above locality records have been in error.

Following are comparisons, descriptions, and figures of *O. aquillae* with *O. talaje* and *O. kelleyi*. The descriptions and figures of *O. talaje* are based on specimens of all stages collected near the type locality in Guatemala. I am grateful to Dr. E. Brumpt of Paris for this material, which includes adults and reared material.

The descriptions and figures of *O. kelleyi* are based on all the materials in the collection of the Rocky Mountain Laboratory, including the types and specimens subsequently collected.

All drawings of *O. aquillae*, *O. talaje* and *O. kelleyi* in figures 2 and 3 were made to conform to the average measurements, so that the figures show relative size.

In describing *O. talaje*, Guérin-Ménéville (1849) quotes from notes of M. Nicolet as follows (translated by Virginia Graybeal):

At Casa Vieja de Gastoya, road from Guatemala to Zacopa (Central America) 15 leagues from Guatemala, May 5, 1847, I was awakened several times in the midst of very profound sleep, by some excruciating bites on my hands and body, and my companion, M. Jules, complained even more than I. At 3 o'clock, irritated by these painful bites, I lighted a candle, and found my hands covered with blood and some spots resembling large lice-bites, which I believed must belong to a particularly large species. My companion told me that we might have been bitten by some wasps lodged in the wall of the house. Having awakened the "arriero" (muleteer) who conducted us, and having told him what we had found, he informed us that we were victims of an animal named "Talaje" which was regarded as a large bug. I then relighted my candle in order to look for this insect, and soon I found this *Argas*, which was nauseating to me. Some were filled with blood, and others had a wrinkled skin. I then recalled having found some on my body during the night and rolling them between my fingers, taking them for some of the ticks with which my mule was covered (au point que) several persons told me that it might die of it.

I took a certain number of these *Argas*, which I put in a tube in order to study them in Paris.

These Talajes inhabit cracks in the walls of old houses. These walls were made of rough bamboo and plugging-mortar. They bite in the manner of bugs, and return to their holes during the day for they are nocturnal.

Nuttall (1908) states, "Habitat: native houses, attacks man."

From the above it is apparent that man is the only known host of *O. talaje* in the type locality.

Measurements of available adults were as follows: largest of six females,  $6.00 \times 4.40$ ; smallest,  $4.95 \times 3.70$ . Average,  $5.54 \times 3.72$ . One available male  $4.85 \times 2.75$ . Average length of many larvae 0.64.

In the adults of *O. talaje* the mammillae are very large in all peripheral areas. The dorsal surface is occupied very largely by the depressed areas which include the large discs, and in which the mammillae are few in numbers. The adult hypostome is very much like that of *O. kelleyi* and differs from both *O. kelleyi* and *O. aquilae* in lacking the short 3d file (median) of denticles.

The larvae of *O. talaje* are the smallest of the three species here compared and have an average measurement of 0.66. The hypostome lacks the anterior projection from which the denticled portion continues.

*O. kelleyi*. This species was originally described from three nymphs taken from bats, and from two cast larvae skins, taken from near the Utah-Colorado State line. Later studies were made of numerous specimens of nymphs and adults taken from houses and other buildings in New York, Wisconsin, Minnesota, Pennsylvania, Illinois, and Iowa—all northern states. All of these buildings were reported to be frequented by bats. Larval studies are from material kindly furnished by Dr. W. A. Riley, which had hatched from eggs laid by a female taken from a house in Minnesota.

The only known hosts of *O. kelleyi* are bats.

Measurements of all available adults are as follows: largest of six females  $7.50 \times 4.00$ ; smallest,  $5.97 \times 3.24$ . Average  $6.83 \times 4.00$ .

The average length of the larva of *O. kelleyi* is 1.09.

Larval hypostome long, very narrow, sides parallel, placed on an anterior extension of the basis. Length of portion with denticles 0.18; length from insertion of the palpi to apex, 0.30.

#### SUMMARY OF COMPARISON OF *O. talaje*, *O. kelleyi* AND *O. aquilae*

Adults of *O. kelleyi* are largest, *O. talaje* intermediate, and *O. aquilae* smallest. Mammillae are relatively largest in *O. talaje* and distinctly fewer in number because the discs and the depressed areas which include them are relatively larger.

The three species show only minor distinctions in tarsus I. That of *O. talaje* is longer and more slender than in *O. aquilae*. Tarsus IV of *O. kelleyi* is distinctly longer than tarsus I. The hypostomes are all very much alike excepting that in *O. aquilae* both sexes show files 3 present.

The larvae of *O. aquilae* are intermediate in size between *O. talaje* and *O. kelleyi*; *O. kelleyi* is largest. The larval hypostomes of *O. aquilae* and *O. talaje* are very much alike but lack the anterior extension from which the barbed portion continues, which is present in *O. kelleyi*.

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# THE RESPIRATION OF THE PROTOZOAN PARASITE, *EIMERIA TENELLA*<sup>1</sup>

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## INTRODUCTION

The economic importance of the protozoan parasite, *Eimeria tenella*, has stimulated a great deal of research along the lines of pathology, specificity, prevention, and its resistance to environmental factors such as temperature and chemicals. Certain of the more academic studies of this parasite have been somewhat neglected. The authors are unaware of research which measured the metabolic rate of the parasite itself or of the tissues invaded by the parasite. It was to supplement our knowledge along this line that work on the metabolic rate of the parasite and invaded tissues was undertaken.

## MATERIALS AND METHODS

*Source of the oöcysts and infected tissues.*—Single Comb White Leghorn chickens supplied by the Department of Poultry Husbandry of the University of Wisconsin were used to supply the oöcysts and tissues for this investigation. Those used to produce oöcysts weighed approximately 700 grams, while those used in studies on the respiration of the coccidia within the epithelial cells of the cecal pouches weighed approximately 300 grams. All chickens were inoculated with approximately 50,000 sporulated oöcysts and were placed in sterile wire cages with outside feed and water containers at the time of infection.

*Preparation of unsporulated oöcysts.* On the 9th day after infection the birds were sacrificed and the cecal pouches removed. The cores and scrapings of the cecal pouches containing the oöcysts were placed in the Waring blender with sufficient water to just cover the blades. From 3 to 5 minutes were found to be sufficient to separate the oöcysts from the cecal material.

At the end of the mixing period a sufficient amount of water was added to increase the volume about 3 times. The resulting mixture was placed in a large separatory funnel and allowed to stand for 30 minutes. The sediment was then transferred to 50-cc centrifuge tubes.

The method of concentrating the oöcysts and washing them free of bacteria was a modification of that used by Lane (1922) for concentrating helminth eggs and by McCoy (1929) for the sterilization of hookworm eggs. The sediment was washed clear of much of the extraneous material by repeatedly centrifuging with top water at a speed of about 1000 revolutions per minute. When the supernatant fluid became clear, saturated sodium chloride solution was added to the sediment and the

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tubes again centrifuged. The resulting surface film containing the oöcysts was transferred to another centrifuge tube by means of a piece of glass tubing or the open end of a small vial. The transferred oöcysts were centrifuged again with sterile distilled water to free them of the concentrated salt solution.

Bacteriological counts of the oöcyst suspension after repeated washing and flotation with salt solution revealed only a small number of bacteria. To free the suspension of the few remaining bacteria, 5 per cent antiformin made up in 10 per cent formalin was added and the mixture allowed to stand for 5 minutes. The oöcysts were again concentrated by centrifuging and the antiformin-formalin solution poured off. To test the sterility of the suspension, 0.1 cc was inoculated into tubes of nutrient agar, which were incubated at 37° C for 24 hours. Only the respiratory determinations on the bacteria-free oöcyst suspensions are included in this report. The time required to prepare the oöcysts and the respiratory apparatus for the initial reading was approximately 4 hours.

*The preparation of sporulated oöcysts.*—The oöcysts to be sporulated were freed from the host tissue by means of the *Waring blender* as described above, and were then placed in a thin layer in Petri dishes and diluted about 5 times with 2.5 per cent potassium dichromate. The Petri dishes were left partially open to maintain adequate oxygen in the solution.

The procedure followed in preparing the sporulated oöcysts for respiratory determinations was much the same as that described for unsporulated oöcysts. The sporulated oöcyst suspension was passed through a 100-mesh copper sieve to remove larger particles. The filtrate was centrifuged several times in water to remove the potassium dichromate solution and most of the extraneous material. Saturated sodium chloride solution was used for oöcyst flotation and 5 per cent antiformin made up in 10 per cent formalin solution was used as described above to kill the bacteria.

*Method of counting the oöcysts.*—To count the oöcysts, 0.1 cc of oöcyst suspension was diluted with 0.9 cc of water. After thorough mixing a drop of the suspension was placed on a Spencer Bright-Line hemacytometer. The oöcysts in 5 of the millimeter squares were counted and the number multiplied by 2 to get the number for 1 cubic millimeter. The final values were all expressed in cubic centimeters.

*The respiratory apparatus, method of calculation, and solutions.*—The Warburg apparatus was used throughout this investigation for respiratory determinations. The method of calculating the data was according to that of Dixon (1934). To calculate the amount of oxygen absorbed in the respiratory flasks from the manometer readings, it was necessary to know the manometric constant for each of the flasks except that used as the thermobarometric flask. These constants were calculated by substituting the proper values in the following equation and simplifying:

$$K_{O_2} = \frac{V_g \frac{273}{T} \text{ plus } V_f a}{P_o}$$

where  $V_g$  is the volume of gas between the surface of the liquid of the flask and the 150-millimeter mark on the manometer,  $V_f$  is the volume of the liquid within the flask,  $T$  is the absolute temperature of the water bath, and  $P_o$  is the pressure of the Brodie solution of the manometers. Once the constant ( $K_{O_2}$ ) for a flask

at a given temperature was known, the rate of oxygen consumption could be readily calculated by use of the following formulas:

$$\text{for oöcyst suspensions: } Q_{O_2} = \frac{hK_{O_2} \times 10^6}{\text{number of oöcysts}}$$

$$\text{for tissue respiration: } Q_{O_2} = \frac{hK_{O_2}}{\text{dry weight of tissue}}$$

where h is the reading of the manometer in cubic millimeters.

Phosphate buffers were used as the substrate in most of the experiments and the different pH intervals prepared by mixing in different proportions, 0.1 M potassium monobasic phosphate and 0.1 M potassium dibasic phosphate. For pH ranges below 4.5, KCl-HCl buffers and phthalate buffers were prepared according to the method described by Clark in his book on the determination of hydrogen-ion concentration.

#### PRESENTATION OF DATA

*The respiration of cecal pouch tissue parasitized by E. tenella.*—To determine the respiratory rate of normal tissues and that of tissues parasitized by *Eimeria*,

TABLE 1.—Showing the difference between the  $O_2$  consumption of normal and parasitized cecal pouch tissue. ( $Q_{O_2}$  = cmm  $O_2$  per mgm of tissue per hour)

Experiment No.	Normal cecal pouch tissue			Parasitized cecal pouch tissue		
	Flask No.	$Q_{O_2}$	Wt. of tissue in mgm	Flask No.	$Q_{O_2}$	Wt. of tissue in mgm
1	2	7.5	4.8	4	10.7	3.4
	3	5.8	5.4	6	6.6	4.4
2	1	4.8	3.0	4	7.8	4.0
	2	3.7	4.0	6	4.3	3.9
3	1	3.2	6.0	7	6.0	3.1
	2	5.5	4.4	4	10.4	6.7
4	1	3.3	6.3	6	7.0	6.3
	2	5.5	11.0	3	7.9	5.2
5	1	4.8	4.5	6	8.7	5.0
	2	3.7	3.0	7	7.4	6.0
Mean		4.7 ± .08		7.3 ± .11		3.1

small, relatively uniform samples were removed from the cecal pouches of the chickens by means of a tonsil punch, 4 millimeters in diameter. The tissue samples were removed as quickly as possible (a few seconds after the chickens were killed), washed in sterile saline, then in a 1 to 1 mixture of a phosphate buffer (pH 7.3) and 1-500 solution of sulfanilamide. Two cc of the same solution were placed in the respiratory flasks to serve as substrate. The temperature of the flasks was maintained at 38° C and readings of the manometers were taken at 30-minute intervals over a period of 2 hours. At the termination of the experiment the tissues were removed to a weighing bottle and reduced to a constant weight in a drying oven. The dry weights were used to calculate the oxygen consumption and all rates were expressed in terms of oxygen consumed per milligram of the dry tissue per hour. Two Warburg flasks containing normal tissues and three containing parasitized tissue were run simultaneously.

The results of experiments to determine the respiratory rate of normal and parasitized tissue are shown in Table 1. The mean  $Q_{O_2}$  for the 10 determinations on

normal cecal pouch tissue was 4.7, and that for the 13 determinations on parasitized tissue was 7.3. The data on the oxygen consumption of both types of tissues were analyzed statistically and found to be significantly different. It must be concluded, therefore, that the parasites had a significant effect on the oxygen consumption of the cecal pouch tissue.

*Normal respiration of oöcysts.*—Normal respiration in this case is that of oöcysts placed under conditions known to be satisfactory for practical laboratory work. When oöcysts are kept at room temperature or slightly above (30° C) and at a pH of approximately 7.3, they sporulate rapidly and uniformly, and may then remain viable for over two years. The temperature of 30° C and pH 7.3 were taken as the normal. The number of oöcysts in each Warburg flask varied among the different experiments and ranged from 3,000,000 to 12,000,000 per cc. The number of oöcysts per flask was the same in any one experiment and the calculations were all reduced to the amount of oxygen per million oöcysts. Determinations were made for varying periods with readings taken at half-hour intervals.

TABLE 2.—*The rate of oxygen consumption of unsporulated oöcysts during the first twelve hours. ( $Q_{O_2}$  = cmm  $O_2$  per million oöcysts per hour)*

Exp. No.	Flask No.	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.	7th hr.	8th hr.	9th hr.	10th hr.	11th hr.	12th hr.	Number of oöcysts
2	1	12.2	10.0	13.0	11.2	9.0	12.0	11.0	8.0	10.0	14.0	20.2	26.0	3,560,000
4	1	20.9	12.7	17.3	15.0	17.3	17.3	15.0	16.1	13.8	23.1	27.7	39.3	3,000,000
4	2	14.5	15.3	16.6	15.0	16.6	15.3	29.3	8.3	14.0	15.3	35.3	38.9	4,000,000
28	1	8.0	6.9	10.2	7.0	7.1	7.2	6.9	6.9	8.0	10.0	18.9	31.0	2,000,000
30	1	18.9	9.4	18.9	14.2	17.8	16.6	14.2	14.2	14.2	13.0	27.3	37.9	2,000,000
30	2	19.2	13.2	18.0	13.8	16.2	18.0	15.6	14.4	14.4	13.2	26.4	38.4	2,000,000
Average		15.6	11.2	16.6	12.7	14.0	14.4	15.3	11.3	12.4	14.7	26.0	35.2	

Data on the unsporulated oöcysts are presented in Table 2 and show that little change in  $Q_{O_2}$  occurred during the first 10 hours. There was however, a great change following this period. The  $Q_{O_2}$  for the first 10 hours ranged from 11.3 to 15.6. During the 11th hour the  $Q_{O_2}$  increased to 26.0 and on the 12th hour was 35.2. This increase is in accord with the findings of George Ott (unpublished data) who found that there was little visible change in the protoplasm of the oöcysts during the first 12 hours, but a marked change during the second 12-hour period. Circumstances beyond control prevented the completion of the  $Q_{O_2}$  determinations between the 12- and 48-hour period. By the time the oöcysts were 48 hours old, the  $Q_{O_2}$  had decreased to about 0.4.

There was considerable variation in the  $Q_{O_2}$  of the various batches of oöcysts but when the data were examined statistically the variation was not significant.

The determinations on the normal rate of respiration of sporulated oöcysts were performed under conditions similar to those for unsporulated oöcysts. The temperature of the water bath of the respirometer was 30° C, the pH of the substrate was 7.3, and the length of the determinations was 3 hours. The concentrations of the oöcyst suspensions used were from 5 to 12 millions. The results of these determinations are given in Table 3. An inspection of the  $Q_{O_2}$  values shown in this table will reveal the marked difference between unsporulated and sporulated oöcysts as to rates of respiration.

The number of oöcysts used in the 23 determinations on sporulated oöcysts may

TABLE 3.—The rate of  $O_2$  consumption of sporulated oöcysts over a period of three hours. ( $Q_{O_2}$  = cmm  $O_2$  per million oöcysts per hour)

Exp. No.	Flask No.	$Q_{O_2}$	No. of oöcysts
21	6	1.00	12,420,000
20	3	0.60	12,300,000
18	1	0.61	11,500,000
14	2	0.23	10,000,000
14a	3	0.16	10,000,000
14b	4	0.23	10,000,000
14c	6	0.33	10,000,000
14d	1	0.20	10,000,000
13	1	0.30	7,920,000
13a	2	0.50	7,920,000
13b	3	0.60	7,920,000
13c	4	0.40	7,920,000
13d	6	0.40	7,920,000
13e	7	0.50	7,920,000
11	1	1.30	7,330,000
10	1	0.38	7,000,000
10a	2	0.56	7,000,000
15	1	0.33	7,000,000
15a	2	0.36	7,000,000
15b	3	0.46	7,000,000
12	1	0.90	6,000,000
16	1	0.26	5,670,000
16a	3	0.46	5,670,000

be considered in 4 classes: (1) 6 to 7 millions, (2) 8 millions, (3) 10 millions, (4) 11 to 12 millions. The average  $Q_{O_2}$  for the 4 classes in their order were 0.5, 0.4, 0.2, and 0.7. These values show on inspection a decrease in oxygen uptake as the number of oöcysts used increased from 6 to 10 million, but above 10 million the rate increased over all other values. Statistical analysis was made of values in two of the number classes to determine the significance of the difference between the mean values of the groups. The two classes selected were the 8 million (Exp. 13) and the 10 million class (Exp. 14). The mean rate for the 10 million class was 0.3 mm<sup>3</sup> per million oöcysts per hour, and the standard deviation was 0.15. With the 8 million the mean was 0.8 and the standard deviation 0.11. When these values were substituted in the proper statistical formulas, no significant difference was revealed between the 2 means. The oxygen uptake of the sporulated oöcysts is so small, and the variations between rates taken at different times is so great that it is probable that a much larger number of determinations will have to be made before any distinct trend in uptake due to numbers can be detected. Some of the possible reasons for the variations seen between runs will be discussed later.

*The effect of pH on oxygen consumption of oöcysts.*—Six experiments were conducted to determine the effect of a given pH range on the oxygen consumption of sporulated oöcysts. Each of the experiments consisted of 3 flasks with 2 cc of phosphate buffer and 1 cc of an oöcyst suspension. The pH intervals used throughout the series were 4.7, 7.3, and 8.8. The method of preparation of buffers at the various pH intervals was according to that described under the section on

TABLE 4.—The effect of pH on the  $Q_{O_2}$  of sporulated oöcysts of *Eimeria tenella*. ( $Q_{O_2}$  = cmm of  $O_2$  per million oöcysts per hour)

Exp. No.	No. of hours observation	Rate of $O_2$ consumption in phosphate buffer at different pH intervals			No. of oöcysts used
		pH 4.7	pH 7.4	pH 8.8	
17	6	0.24	0.22	0.22	7,000,000
18	6	0.72	0.64	0.71	11,500,000
19	6	0.82	0.80	0.93	16,000,000
20	6	0.22	0.20	0.20	12,300,000
21	6	0.96	0.92	0.90	12,420,000
22	6	0.43	0.44	0.40	18,000,000



materials and methods. Each of the experiments ran for 6 hours and the  $Q_{O_2}$  values, which are given in Table 4 represent averages of the rates of oxygen consumption for the total length of each experimental run.

Because of the large amount of variation in rates of oxygen uptake between determinations made at different times and on different oöcyst suspensions, any conclusions drawn as to the effect of pH on the rates of oxygen consumption must be based on comparisons of the values within each experiment. Some of the differences may be due to differences in the number of oöcysts in the various experiments and some may be due to some physiological differences in the oöcysts themselves at the time of the experiments. In spite of these differences, the outstanding fact is that the pH values within the range considered had no significant effect on the rate of respiration of sporulated oöcysts.

Since sporulated oöcysts exhibited such a high resistance to the effects of pH, a characteristic resistance which these organisms show toward other chemical factors, it was surmised that a different type of response might be produced with unsporulated oöcysts. To test this hypothesis an experiment was performed with

TABLE 5.—The effect of pH on the respiration of unsporulated oöcysts

Flask No.	Type of substrate	pH of substrate	Rate of $O_2$ consumption in $ccm/10^6/hr.$	Number of oöcysts
1	KCl-HCl	1.3	16.0	2,000,000
2	Phthalate	2.4	16.4	2,000,000
3	$PO_4$ -buffer	4.7	15.6	2,000,000
4	$PO_4$ -buffer	7.3	15.3	2,000,000
5	$PO_4$ -buffer	8.8	15.6	2,000,000

unsporulated oöcysts in substrates at 5 different pH intervals. The substrates used were KCl-HCl buffer at pH 1.3, phthalate buffer at pH 2.4, and phosphate buffers at pH 4.7, 7.3, and 8.8. The data (Table 5) reveal that differences in rates are too small to be attributed to differences in pH of the substrates. On the basis of all experiments presented it must be concluded that pH of the substrate within the limits tested has no effect on the respiration of oöcysts which can be detected by the methods used in this investigation.

#### DISCUSSION

*Respiration of normal and parasitized tissue.*—When the epithelium of the cecal pouches of chickens infected with *Eimeria tenella* is examined it is not surprising to find a significant increase in the respiratory rate of the parasitized tissue. The parasitized cells are greatly enlarged; the submucosa is packed in many areas with displaced epithelial cells and the small capillaries normally present have become greatly distended with blood. The general picture is that of an inflammatory reaction. While it is not difficult to imagine an increase in metabolism of the parasitized tissue, it is not easy to explain just how much of the increase is due directly to the parasites themselves and to the increase due to the pathology of the tissues. It was not the purpose of this study to determine the cause of the increase but rather to measure the change in the metabolic rate of the parasitized cells if a change did occur.

Since the tissues studied were not completely freed of bacteria, the effect of these organisms on the results of the experiments must be considered. The possibility

that the bacteria were responsible for the difference in the metabolic rate of the parasitized and normal tissues seems unlikely. The tissues were prepared in exactly the same manner, and George Ott (unpublished data) found no significant change in the number of types of bacteria present during the development of coccidiosis.

*Respiration of sporulated and of unsporulated oöcysts.*—In the unsporulated oöcysts there is a rounded mass of undifferentiated protoplasm. During the first 24 hours following discharge of the oöcysts into a favorable external environment, the oöcyst undergoes a division into 4 spores each containing 2 sporozoites. Within 48 hours practically all of the visible oöcysts have undergone complete development and have entered the resting stage with a resulting greatly reduced metabolic rate.

When the data on the metabolism of the 2 stages of oöcysts are examined the difference in  $Q_{O_2}$  is obvious. It is also evident that there existed a wide variation in the metabolic rate of oöcysts produced by different groups of chickens. No attempt will be made to explain this variation. That point alone may require years of concentrated study to solve. There are, however, several factors which can be mentioned that may throw some light on the subject.

The oöcysts are the product of the second generation merozoites which are released on the 5th day following infection. With this release there is a sudden and great decrease in the number of erythrocytes due to hemorrhage (Herrick, Ott, and Holmes, 1936). There is also a drop in hemoglobin, a great decrease in non-protein and total nitrogen, and concomitant with these changes, there is a great increase in blood sugar (Pratt, 1940, and Waxler, 1941) and blood chlorides (Waxler, 1941). It is during the 6th and 7th day following infection that the temperature may drop several degrees and the greatest mortality occurs. The mortality from coccidiosis may reach 100 per cent but that is unusual even from heavy artificial infections. In nearly every infection some chickens recover. It is only those that pass through the various pathological changes and recover that ever produce oöcysts. It is conceivable, therefore, that any one of the factors mentioned may affect the metabolism of the resulting oöcysts. It has been discovered by S. A. Edgar (to be published soon) that late on the 6th day, the macrogametocytes store relatively large quantities of glycogen. Undoubtedly other materials are stored at this time for use in the later sporulation process. It has been shown that canaries infected with malaria are more severely affected if fed sugar (Hegner and MacDougall, 1926), and rats infected with some pathogenic trypanosomes survive longer if starved or injected with insulin than if given normal treatment and feed. It is also reasonable to think that chickens which exhibit a greater resistance to coccidiosis may greatly affect the physiology of the oöcysts produced. It is conceivable that the amount of blood sugar in the blood of the chicken, the nitrogen level, or the amount of hemoglobin in the blood could affect the amount or quantity of materials stored by the gametocyte and thus affect their metabolic rate during subsequent development.

It is soon learned by anyone working with coccidian oöcysts that certain batches remain viable for long periods, two years or more, while others lose their infectivity within a few months. Whatever factor or factors affect the viability of the oöcysts may also cause the variation in metabolism observed among oöcysts secured from different chickens. Such explanation must await future work.

*The effect of pH on respiration.*—Cases in which the respiratory rates have been

shown to vary in substrates of different pH have been reported for several phyla. Hiestand and Hale (1938) found the respiratory rate of fresh-water molluscs to decrease when the pH was lowered. Hiestand (1940) found the respiratory rate of *Thyone* (Holothurian) to increase as the pH increased from 5.4 to 8.8. Von Brand (1943) found that oxygen consumption of *Eustrongylus* (nematode) remained practically unchanged in the pH range from 3.4 to 8.3. An increase in oxygen consumption was, however, observed in the two pH ranges 1.1 to 2.0 and 9.0 to 10.7. Maier and Coggeshall (1941) found that the respiratory rate of *Plasmodium knowlesi* remained constant between pH 7.0 and 8.0 but declined with further increase in pH up to 9.0.

In the present study no significant change in the rate of respiration of unsporulated oöcysts within the pH range of 1.2 to 8.8 and of sporulated oöcysts within the pH range of 4.5 to 8.8 was observed. These results are in accord with the findings of Horton-Smith and others (1940) who found that the unsporulated oöcysts of *E. tenella* could withstand exposure to sodium hydroxide (pH 11.2) for 2 hours and concluded that the effects of ammonia on *E. tenella* were due to toxic action of the drug rather than to effect of the change in hydrogen-ion concentration.

#### SUMMARY

1. The oxygen consumption of tissue parasitized with *E. tenella* was significantly higher than that of normal tissue.
2. The respiration of unsporulated oöcysts is from 10 to 20 times greater than that of sporulated oöcysts.
3. The respiration of oöcysts remained fairly constant within the pH range of 1.2 to 8.8.

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## AMERICAN VISCERAL LEISHMANIASIS— THE ETIOLOGICAL AGENT\*

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### INTRODUCTION

Migone (1913) reported a case of visceral leishmaniasis from Paraguay, in an Italian who came to Brazil in 1899. In February 1911 the patient became ill with chills, fever, diarrhea and lassitude and was treated with quinine. In May he went to Asuncion, where he was found to be suffering from loss of strength and weight, anemia, leukopenia, spots on the face, back and hands, spleno-hepatomegaly, pot-belly, coated tongue, and epistaxis. *Leishmania donovani* was found in blood smears. He was treated with 0.6 gm salvarsan but died a month later with a severe attack of diarrhea. It is very likely that this patient acquired the infection in Brazil because he became sick twenty years after leaving Italy. Mazza and Arias (1926) reported autochthonous infantile kala-azar in Salta, Argentina, while Chagas and Romãña (1937) found cases in Chago, Argentina.

Penna (1934) reported that the Viscerotomy Service of the International Health Division of the Rockefeller Foundation on Yellow Fever cooperating with the National Department of Health of Brazil between March 1932 and July 1936 found 85 specimens of liver containing leishmania bodies morphologically identical with those of *Leishmania donovani*. These cases were in the states of Bahia, Sergipe, Alagôas, Pernambuco, Rio Grande do Norte, Ceará, Piauí and Pará. Gatti, Boggino and Prieto (1939) reported an adult Paraguayan who developed kala-azar in Bolivia. Martinez and Pons (1941) described the first case of an adult with kala-azar in Venezuela. Later, Patenza and Andeze (1942) examined 842 liver specimens removed by viscerotomy and found a child infected with *Leishmania*. Cases have been found in persons between the ages of 45 days and 56 years, of whom 53.1 per cent were under 6 years, 17.4 per cent between 6 and 10 years and 29.5 per cent over 10 years of age. No evidence of epidemic incidence of the infection was found in any focus, but the disease occurs endemically and is apparently a jungle infection. The mortality was 1.8 per cent in the Amazon Valley and 0.4 per cent in the North-eastern section of Brazil.

Clinically, the evolution of the disease is very similar to that of Indian and Mediterranean kala-azar. The onset is variable, with progressive anemia, emaciation, leukopenia, monocytosis, spleno-hepatomegaly, and hemorrhagic manifestations. Death in acute cases takes place in one to three months and in chronic cases in 8 to 15 months. The parasites are easily detected in blood and by spleen and liver puncture. Cultivation on N.N.N. medium gives rise to leptomonad forms. Treatment with antimony preparations has proven specific (Chagas, 1936).

The report of the American Kala-Azar Commission (Chagas *et al*, 1938) deals with eight cases in the state of Pará in 1937, seven of which were children. Of 1,446 animals examined, seven dogs and a cat were found infected.

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Chagas *et al* (1937) claimed that by means of serological tests with immune sera, prepared by inoculating cultures into animals, the South American parasite could be differentiated from the other species of *Leishmania* and that on this account the name "*L. chagasi*" given to the parasite is appropriate. It would appear, however, that the differences noted are not greater than those which have been observed to exist among different varieties of the same species in China, India, the Sudan and the Mediterranean area. The statement that animals cannot be infected is untrue in view of the fact that da Cunha (1938, 1938a, 1938b) was able to infect the hamster, rhesus monkey and the dog, and Adler (1938) was able to infect the Syrian hamster with culture material. Another argument, namely, that the South American disease is claimed to be endemic in character in contrast to kala-azar of the Old World which is epidemic, is also invalid, since the Old World kala-azar is rarely epidemic.

Da Cunha (1938) carried out a number of serological tests with strains of *L. donovani*, *L. infantum*, *L. tropica*, *L. brasiliensis* and the *Leishmania* of South American kala-azar. Agglutinating sera were prepared against these organisms in rabbits. With these sera and cultures of the various strains, agglutination-absorption tests were made which indicated that "*L. chagasi*" is identical with *L. infantum* (of Mediterranean kala-azar).

Ferreira, Mangabeira, Deane and Chagas (1938) demonstrated that *Phlebotomus intermedius* became infected when fed on an infected dog, and Chagas (1939) succeeded in infecting *P. intermedius* and *P. longipalpis* by allowing them to feed on an infected dog. Later, Paranse and Chagas (1940) demonstrated that such artificially infected sandflies when emulsified and injected intraperitoneally into the hamster gave rise to generalized leishmania infection.

Adler and Theodor (1939) infected *P. papatasi* with the organism of American kala-azar by feeding the fly on suspensions of the cultured organisms. An infection rate of 24 per cent was produced in sandflies fed on culture containing 300 flagellates per 0.1 cu mm, 71 per cent in sandflies fed on emulsions of 1000-2000 per 0.1 cu mm and 89 per cent in higher concentrations. The flagellates adopted an anterior position in the sandflies.

The writer has studied the biochemical reactions of representatives of the genus *Leishmania* and of *Trypanosoma cruzi*. On the basis of the nature of their growth *L. tropica*, *L. donovani*, *L. brasiliensis* and *T. cruzi* were found to be eugonic while the dysgonic forms were *L. caninum* and *L. infantum*. All the species failed to ferment the mono- and polysaccharides, failed to produce indol, and methyl red and Voges-Proskauer tests were negative. *L. tropica* and *T. cruzi* grew in milk, failed to produce  $H_2S$  in lead acetate medium and did not liquefy gelatin. The remaining leishmanias failed to grow on these media. The thermal death point for all the leishmanias was at 40° C in 15 to 30 minutes and at 45° C immediately, while for *T. cruzi* at 40° C in 3½ hours and at 45° C in 15 to 30 minutes. *L. brasiliensis* was insoluble in bile in 7 days, while *T. cruzi* was soluble in 96 hours, but the rest of the leishmanias were soluble in 24 hours. Colonial growth was obtained on the surface of the medium when *L. tropica* and *T. cruzi* were grown on the *Leishmania* medium (Senekjie, 1939, 1939a, 1943; Senekjie and Zebouni, 1941).

#### STATEMENT OF THE PRESENT PROBLEM

The object of the present study has been to ascertain the biochemical reactions,

the physical properties and the cultural characteristics of the etiological agent of American visceral leishmaniasis, and its relationship to the etiological agent of Old World kala-azar.

#### MATERIAL AND METHODS

The writer wishes to express his thanks to Dr. Emanuel Dias of Instituto Oswaldo Cruz of Rio de Janeiro who kindly sent cultures of "*L. chagasi*."

Leishmania-blood-agar, leishmania serum, carbohydrate medium, methyl red, Voges-Proskauer, indol, lead acetate, and milk media were prepared according to the writer's previous formulae (Senekjie, 1939, 1943; Senekjie, and Zebouni, 1941) but rabbit defibrinated blood or serum was used exclusively throughout these experiments.

The thermal death point was determined by heating saline suspensions of week-old cultures of "*L. chagasi*" in a water bath at 40° and 45° C and making direct microscopic observations for motility, and cultures at intervals of 5, 15, and 30 minutes.

The bile-solubility tests were performed by using sterile human bile obtained from the normal gall-bladder post mortem. Three-tenths cc of sterile human bile was added to 1 cc of a saline suspension of week-old cultures of "*L. chagasi*." Control cultures containing the organisms in physiologic saline solution were also prepared. The tubes were kept at room temperature, and direct microscopic observations were made at 15-, 30-, 45-, 60-minute, and at 18- and 48-hour intervals.

The egg-liver extract medium was prepared according to the formula of the writer (Senekjie, 1943).

#### RESULTS

*Growth on carbohydrate media.* The growth of "*L. chagasi*" became apparent in five days after incubation at room temperature. At first the growth was finely granular, but in 10-15 days the clumps became quite large and settled to the bottom of the tube, leaving a clear supernatant layer. On shaking the tubes, the clumps were broken up, and the suspension again became finely granular. No acid or gas was produced. The growth was eugonic. The following carbohydrates were tested in the culture media: dextrose, maltose, lactose, sucrose, mannite, dulcitol, rhamnose, xylose, sorbitol, levulose, galactose, and inulin.

"*L. chagasi*" did not produce indol and the H<sub>2</sub>S, methyl red and Voges-Proskauer tests were negative. In milk there was no growth.

*Thermal death point.* Table 1 shows the thermal death point of "*L. chagasi*." At 40° C the organism was killed in 30 minutes, but at 45° C it was killed immediately.

TABLE 1.—Thermal death point of "*L. chagasi*"

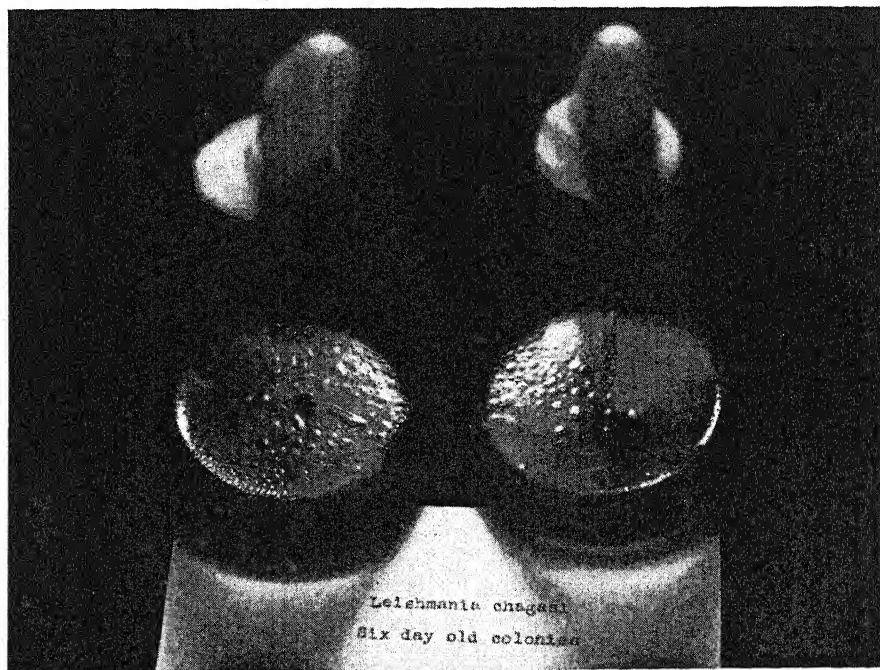
Time	40° C		45° C	
	Direct	Culture	Direct	Culture
5 min.	++	++++	—	±
15 "	—	++	—	—
30 "	—	—	—	—

*Bile solubility.* Table 2 shows the bile solubility of the organism. It was completely dissolved in 24 hours, while the saline controls remained motile for about six days.

TABLE 2.—Bile solubility of "*L. chagasi*"

Time	Bile	Saline
15 min.	++++	++++
30 "	++++	++++
45 "	++++	++++
60 "	++++	++++
18 hours	++	++++
24 "	-	++++
48 "	-	++++
6 days	-	+++

*Colony characteristics.* In 24 hours a surface film appeared. About the third day small colonies about 2 mm in diameter developed; these had an irregular contour, were slightly elevated, with a moist, shiny surface, a finely granular structure, amoeboid edges, and dirty white, transparent appearance. In about a week, the size was 3–6 mm, the colonies were irregular in shape, convex, moist, shiny and finely granular, with amoeboid "pseudopods." The colonies stopped growing about the tenth day, but progressively the surface became wrinkled, and if drying was prevented the organisms remained alive for about three weeks.



*Egg-liver extract medium.* "*L. chagasi*" grew well on this medium; about the fourth day the medium developed turbidity, which increased until about the tenth day. There was no surface film, but some sediment accumulated at the bottom of the slant. Usually the growth was smooth, but at times finely granular. Under the microscope longitudinal binary fission was frequently observed. Such cultures survived two weeks. By serial subcultures the organisms lived for about six generations, then stopped growing. Apparently this was due to progressive consumption of the stored growth fraction of the media. When such a scanty culture was transferred to a blood medium, the organisms grew abundantly. It was also observed

that "*Leishmania chagasi*," and *Trypanosoma cruzi* grow well on egg-medium base with physiologic saline or Ringer's solution overlay in place of 0.5 per cent liver extract in saline.

#### DISCUSSION

Visceral leishmaniasis has a very wide geographical distribution. The Chinese type occurs mostly among small children but infection of adults is not rare. The dog has been proved to be a reservoir host, and the transmitting vectors are *Phlebotomus chinensis* and *P. sergenti*. In India 39½ per cent of the patients are less than 10 years old. No reservoir host has been discovered, but the vector is *P. argentipes*. The infantile kala-azar of the countries around the Mediterranean Sea mainly occurs in children below 4 years. The dog is the important reservoir host, and the vectors are *P. perniciosus*, *P. major*, and *P. papatasi*. The most characteristic feature of the Sudanese type is that it is resistant to treatment with antimony. The vector is *P. langeroni*. As previously stated, New World or American visceral leishmaniasis occurs in infants and adults from 45 days to 56 years of age. The reservoir host is the dog and sometimes the cat, while the vectors are *P. intermedius* and *P. longipalpis* (Ferreira *et al*, 1938; Chagas, 1939).

The writer is inclined to believe that *L. donovani*, the causative agent of the Old World visceral leishmaniasis and "*L. chagasi*," that of American visceral leishmaniasis, are identical because of the following findings:

1. Both diseases are endemic, although Old World kala-azar may at times become epidemic (Chagas, 1936).
2. Sandflies are the intermediate hosts and transmitting agents of all types of *Leishmania*. *P. papatasi*, the transmitter of the Mediterranean type, is infectible with the American type (Adler and Theodor, 1939).
3. Both diseases produce generalized infection in the hamster, rhesus monkey and dog (da Cunha, 1938, 1938a, 1938b; Adler, 1938).
4. The blood picture and blood chemistry are identical in both infections (Chagas, 1936).
5. Agglutination absorption tests show that the two organisms are identical (da Cunha, 1938c).
6. The biochemical, cultural and growth requirements, as well as bile solubility and thermal death point are the same for *L. donovani* and "*L. chagasi*" (Senekjie and Zebouni, 1941).
7. The clinical course of the infection is similar in all types of kala-azar.
8. Antimony salts are specific for all types except that in the Sudan. (Kirk and Sati, 1940; Chagas, 1936).

Since "*L. chagasi*," the etiologic agent of American visceral leishmaniasis, is epidemiologically, clinically, parasitologically, immunologically and culturally identical with *L. donovani*, the agent of Old World kala-azar, *L. chagasi* becomes a synonym of *L. donovani*; hence the former name should not be employed to designate the *Leishmania* producing American kala-azar.

#### SUMMARY AND CONCLUSION

1. "*L. chagasi*" is a eugonic organism which does not ferment carbohydrates, does not grow in milk, does not produce H<sub>2</sub>S or indol, and is methyl red and Voges-Proskauer negative.



2. It is bile soluble, and is killed instantaneously at 45° C and in 30 minutes at 40° C.
3. The colony characteristics are described.
4. Evidence is provided supporting the view that "*L. chagasi*" is synonymous with *L. donovani*; hence *L. donovani* should be employed to designate the causative organism of American kala-azar.

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LYMNAEID SNAILS AS SECOND INTERMEDIATE HOSTS OF THE  
STRIGEID TREMATODE, *COTYLURUS FLABELLIFORMIS*  
(FAUST, 1917)<sup>1</sup>

W. W. CORT, STERLING BRACKETT,<sup>2</sup> AND LOUIS OLIVIER<sup>3</sup>

INTRODUCTION

From 1932 to 1941 a series of studies has been made during the summers at the University of Michigan Biological Station on the second intermediate host relations of the strigeid trematode, *Cotylurus flabelliformis*.<sup>4</sup> The adults of this species live in ducks. The cercariae develop in elongate daughter sporocysts in the digestive gland of the snail intermediate hosts. They escape and penetrate into snails that serve as second intermediate hosts, normally being localized in the hermaphroditic gland inside the digestive gland. In the development of the metacercaria the body of the cercaria goes through a profound metamorphosis and finally becomes an encysted tetracotyle. The developmental stages of the metacercaria of *C. flabelliformis* have never been described in detail but they appear to be like those of the closely related European species, *C. cornutus*, which have been described and figured by Szidat (1924) and Wesenberg-Lund (1934, Pl. XXV). When the cercaria penetrates into the snail it loses its tail and the body soon begins to enlarge. In about 10 to 14 days at favorable temperatures the metacercaria has grown to five or six times the length of the cercarial body, reaching about 1 mm in length, and has become very broad and thick. During this growth the internal structures appear to break down and the stellate cells of the parenchyma are widely separated by fluid-filled spaces. After maximum size is reached the tissues begin to be reorganized and condensed, the size decreases, and the hold-fast organ, the lateral cups and other structures of the tetracotyle are formed. During this reorganization the cystogenous glands become prominent, and a narrower hind-body is set off from the fore-body. Finally, the metacercaria becomes encysted and the development appears to be complete. This development takes about three weeks or more, depending on the temperature. The fully developed metacercaria (tetracotyle) is about one-third the length of the largest developing stage and is surrounded by a secreted cyst.

In order to compare the extent of development in our experiments the metacercariae counted were frequently classified into three arbitrary groups according to their stage of development, viz., (1) "developing" (all stages up to the largest and those beginning to reorganize, (2) "pre-cysts" (those with a distinct hind-body but

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<sup>1</sup> From the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is the fourth of a series on the second intermediate host relations of *C. flabelliformis*. The first was by Winfield (1932), the second by Nolf and Cort (1933), and the third by Cort, Olivier and Brackett (1941).

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<sup>4</sup> For a discussion of the literature on the life cycle of this species see Cort, Olivier, and Brackett, 1941.

without a cyst wall), and (3) "cysts" (those that had completed their development and were surrounded by a cyst wall).

The earlier papers of this series have dealt primarily with the influence of infections of the sporocysts and rediae of trematodes in snails on their second intermediate host relations to *C. flabelliformis*. The present paper will consider the development of the metacercariae in the lymnaeid snails of the Douglas Lake region when they harbor no infections with other larval trematodes. It is especially concerned with the presentation of the evidence that the cercariae of *C. flabelliformis* that develop in the varieties of *Lymnaea stagnalis* differ greatly in their second intermediate host relations from those that develop in the varieties of *Stagnicola emarginata*.

In our examinations of snails at the University of Michigan Biological Station we have found that three species of lymnaeids serve as intermediate hosts for the development of the cercariae of *C. flabelliformis*. Infection is widespread in the region, but usually in low incidence in *Lymnaea stagnalis appressa* Say, *L. s. perampla* Walker,<sup>5</sup> *Stagnicola emarginata angulata* (Sowerby), and *S. e. canadensis* (Sowerby). Single individuals of *Stagnicola palustris elodes* (Say) have also been found infected in one particular area in two separate years.

The various species of lymnaeid snails in the region serve as second intermediate hosts for the development of the metacercariae of *C. flabelliformis*. These metacercariae are widespread and abundant in natural infections in the two varieties of *L. stagnalis* and in *S. e. angulata* and *S. e. canadensis*. In one area they were also found in large numbers in *S. p. elodes*. Also, natural infections were found in at least one of the few collections we were able to make of the huge lymnaeid, *Bulinnea megasoma* (Say). All these species of snails have also been infected experimentally with the metacercariae. In addition, we obtained normal development of the metacercariae in experimental infections of *Stagnicola exilis* (Lea) and *Fossaria abrusa* (Say). The failure to find natural infections in these two species was probably due to the fact that our collections of them were from very limited areas. All these species of lymnaeid snails appear to be normal second intermediate hosts for *C. flabelliformis*, since development of the metacercariae was completed in them. They belong to four different genera of the Lymnaeidae and differ greatly in size, structure and habits. This would suggest that possibly any lymnaeid species might serve as a second intermediate host for this trematode. On the other hand, physid and planorbid snails, appear not to be normal second intermediate hosts for *C. flabelliformis* since development of the metacercariae in them only occurs when they are infected with the sporocysts or rediae of trematodes (Cort, Olivier and Brackett, 1941).

Since the species of lymnaeid snails in this region differ so much from each other it seemed of interest to compare in experimental infections the development of the metacercaria of *C. flabelliformis* in them. In setting up the experiments, examinations for natural metacercarial infection were first made of some of the snails of the collections to be used. The results of these examinations are listed under the descriptions of Tables 1 and 2 in which the results of the experiments are summarized. In carrying out the individual experiments, the snails to be infected were placed in an aquarium, and water containing cercariae of *C. flabelliformis* that had

<sup>5</sup> These two varieties of *L. stagnalis* are difficult to distinguish and are intermingled in the Douglas Lake region. They were used in the experiments without any attempt being made to determine to which variety each individual snail belonged.

escaped from infected intermediate hosts was poured in with them. The numbers of cercariae used and the extent of exposure varied, since frequently the cercariae were poured in over a period of several days. No attempt was made to count the cercariae that were used in infecting any particular group of snails. Therefore, the extent of exposure to cercariae could only be measured by the number of metacercariae that developed. In most of the experiments two different species of lymnaeid snails were exposed together for comparison. Also, in some of the experiments reported in this paper, snails with known infections of larval trematodes were included. The tables included in the present paper, however, give only the results for the unparasitized snails of such experiments. In every experiment some of the snails died before the date of examination. These are omitted from the tables. The experiments were terminated from about 10 days to a month or more after last exposure to the cercariae. The experiment numbers given in Tables 1 and 2 are taken from our original records and are chiefly significant in identifying experiments that are considered in more than one connection.

It was found early in the work that there are certain differences in second intermediate host relations between the cercariae of *C. flabelliformis* that develop in the varieties of *L. stagnalis* and those that develop in the varieties of *S. emarginata*. In an attempt to make these differences clear, those experiments in which the cercariae used in the experimental infections were from *L. stagnalis* are summarized in Table 1 and those in which they were from *S. emarginata* are set up separately in Table 2. The results of the experimental infections for each species of snail will be discussed separately beginning with *L. stagnalis*.

#### A. THE VARIETIES OF *L. stagnalis* AS SECOND INTERMEDIATE HOSTS FOR *C. flabelliformis*

The varieties of *L. stagnalis* were found naturally infected with the metacercariae of *C. flabelliformis*, often with large numbers, wherever snails of this species were present harboring the sporocysts and cercariae of this trematode. In fact, this species of snail might be considered as the type first and second intermediate host for

#### EXPLANATION OF TABLE 1

A summary of the counts of metacercariae of *C. flabelliformis* in experiments in which lymnaeid snails were experimentally infected with cercariae of this trematode species that had developed in the varieties of *L. stagnalis*. All experimental snails were uninfected with larval trematodes.

Control examinations were made as follows for previously acquired natural infections with metacercariae of *C. flabelliformis* of snails from the same collections as those used in the experiments:

- 3A(1935) and 3B(1935): 6 *L. stagnalis*, negative; 6 *S. exilis*, negative.
- 3(1937): 15 *L. stagnalis*, negative; 15 *S. exilis*, negative.
- 6A(1935): 6 *L. stagnalis*, negative; 12 *S. e. angulata*, negative.
- 13(1935): 15 *L. stagnalis*, negative; *S. e. angulata*, no examinations.
- 5B, C, D, E (1937): 50 *L. stagnalis*, 47 negative, 3 positive with 5 "cysts"; 23 *S. e. angulata*, 22 negative, 1 positive with 1 "cyst."
- 4(1937): 20 *S. p. elodes*, 14 negative, 6 positive with 9 metacercariae.
- 2(1941): 3 *B. megasoma*, negative.
- 4(1935): 8 *Fossaria abrusa*, negative.
- 21(1938): 25 juveniles of *S. e. canadensis*, negative.
- 1A and 1B(1941): 20 juveniles of *S. e. angulata*, negative.
- 2(1941): natural infections of metacercariae not found in a number of juveniles of *S. e. angulata* examined from the area from which this collection was taken.



TABLE 1

Exper. No.	Days from in- fection to examination	Species of snails experimentally infected	No. of snails	No. pos. for meta- cercariae	Data from metacercarial counts				
					Range in No.	Av. No.	Per cent "cysts"	Per cent "precysts"	Per cent "developing"
3A (1935)	25-56	<i>L. stagnalis</i>	14	14	828-3092	1742	.....	.....	...
3B (1935)	24-56	<i>S. exilis</i>	5	5	147-397	208	.....	.....	...
	35-36	<i>L. stagnalis</i>	14	14	44-444	168	97.7	1.8	0.5
3 (1937)	"	<i>S. exilis</i>	12	12	11-30	16	100.0	0.0	0.0
	17-23	<i>L. stagnalis</i>	14	14	29-289	159	88.4	10.8	0.8
6A (1935)	"	<i>S. exilis</i>	22	22	3-39	22	75.5	9.0	15.5
	7-29	<i>L. stagnalis</i>	8	8	103-1494	693	.....	.....	...
13 (1935)	7-23	<i>S. e. angulata</i>	5	4	0-21	15	.....	.....	...
	8-17	<i>L. stagnalis</i>	5	5	90-394	214	.....	.....	...
5B (1937)	18-19	<i>S. e. angulata</i>	21	21	1-71	25	.....	.....	...
5C (1937)	"	<i>L. stagnalis</i>	6	6	18-240	103	4.5	27.9	67.6
	26-27	<i>S. e. angulata</i>	10	10	3-51	22	48.4	30.9	20.7
5D (1937)	"	<i>L. stagnalis</i>	3	3	59-158	98	74.6	22.0	3.4
	22-25	<i>S. e. angulata</i>	5	5	3-55	21	21.9	41.9	36.2
5E (1937)	"	<i>L. stagnalis</i>	5	5	75-104	93	73.0	12.5	14.5
	25-33	<i>S. e. angulata</i>	8	8	1-47	23	38.7	25.7	35.6
4 (1937)	"	<i>L. stagnalis</i>	5	5	103-221	158	.....	.....	...
	50-53	<i>S. e. angulata</i>	7	6	0-32	7	.....	.....	...
2 (1941)	16-18	<i>S. p. clodes</i>	35	35	6-108	43	66.5	19.5	14.0
4 (1935)	23-40	<i>B. megalosoma</i>	7	7	3-51	16	0.0	0.0	100.0
21 (1938)	36-37	<i>F. abrusa</i>	23	23	1-87	33	.....	.....	...
1A (1941)	"	<i>S. e. canadensis</i> (juv.)	76	47	0-184	23	.....	.....	99+
1B (1941)	22	<i>S. e. angulata</i> (juv.)	26	0	14-127	49	64.9	35.1	0
	"	<i>S. p. clodes</i>	4	4	6	...	...	...	...
2 (1941)	29	<i>S. e. angulata</i> (juv.)	25	1	21-80	38	95.8	4.2	0
	10-18	<i>S. p. clodes</i>	5	5	0-48	18	0	0	100.0
	"	<i>B. megalosoma</i>	23	18	3-51	16	0	0	100.0

TABLE 2

Exper. No.	Days from infection to examination	Species of snails experimentally infected	No. of snails	No. pos. for meta- cercariae	Data from metacercarial counts				
					Range in No.	Av. No.	Per cent "cysts"	Per cent "precysts"	Per cent "developing"
2 (1936)	22-24	<i>L. stagnalis</i>	39	14	0-13	2	0.0	0.0	100.0
10A (1935)	"	<i>S. cellis</i>	20	20	75-208	124	36.4	18.9	44.7
10B (1935)	25	<i>L. stagnalis</i>	9	4	0-125	17	100.0	0.0	0.0
14 (1935)	24	<i>S. e. angulata</i>	16	16	15-414	137	1.3	98.6	0.0
15 (1935)	8-18	<i>S. cellis</i>	17	17	40-589	146	28.3	71.7	0.0
5 (1936)	0-12	<i>L. stagnalis</i>	5	0	...	...	...	...	...
1 (1936)	28-40	<i>S. e. angulata</i>	15	14	0-464	199	0.0	0.0	100.0
12 (1935)	57-59	<i>L. stagnalis</i>	6	6	4-96	54	0.0	0.0	100.0
11 (1935)	20-24	<i>S. e. angulata</i>	19	19	81-785	374	0.0	0.0	100.0
20 (1938)	24	<i>L. stagnalis</i>	9	4	0-300	40	77.9	6.6	15.5
	"	<i>S. e. angulata</i>	6	6	279-412	302	72.9	8.6	8.5
	"	<i>S. e. angulata</i>	6	6	22-443	278	0.0	0.0	100.0
	"	<i>F. abrusso</i>	2	2	44 & 62	...	0.0	0.0	100.0
	35-36	<i>S. e. canadensis</i> (juv.)	30	28	0-48	12	0.0	0.3	99.7
			58	35	0-131	25	53.3	19.1	27.6

this trematode species, since the first description of its cercaria was from this host (Cort and Brooks, 1928) and the most complete experimental demonstration of its life cycle was made with cercariae from this host experimentally introduced into snails of this same species (Van Haitsma, 1931).

### 1. Infections with *Cercariae* from *L. stagnalis*

Our experiments (Table 1) showed that the metacercariae develop in large numbers in the varieties of *L. stagnalis* when they are exposed to cercariae of *C. flabelliformis* from this same snail species. The numbers that developed were always very much larger than those in the smaller lymnaeids in the same experiments [cf 3A and 3B (1935); 3 (1937); 6A (1935); 13 (1935); 5B, 5C, 5D and 5E (1937)] that had had the same exposure to the cercariae. While these differences might be interpreted as showing that *L. stagnalis* was inherently more susceptible to this infection than the smaller lymnaeids, there are obviously other factors involved. In the first place, the varieties of *L. stagnalis* used in the experiment were several times larger than the two species to which it was directly compared, *S. exilis* and *S. e. angulata*. Also, when snails of this species are irritated by the penetration of the cercariae they tend to extend their bodies out from the shell thus increasing the surface exposed to the attack of the cercariae. The results of the experiments listed in Table 1 also showed a rapid development of the metacercariae in *L. stagnalis*. In some of the snails a few "cysts" were present as early as 16 and 17 days after first exposure to the cercariae. In light and moderate infections most of the metacercariae were in the "cyst" or "pre-cyst" stages by 21 to 27 days after infection [3 (1937); 5C and 5D (1937)]. In heavier infections development was considerably slower as shown in one experiment [3A (1935)] in which only a very small proportion of the metacercariae had become encysted by 27 days and a large proportion were still in the "developing" stages as long as 38 to 56 days after exposure to the cercariae (Table 3).

It can be seen, therefore, that the varieties of *L. stagnalis* are excellent second intermediate hosts for *C. flabelliformis* when they are infected with the cercariae that have developed in the same species of snail. Development is rapid and large numbers of metacercariae can be harbored by a single individual.

### EXPLANATION OF TABLE 2

A summary of the counts of metacercariae of *C. flabelliformis* in experiments in which lymnaeid snails were experimentally infected with cercariae of this trematode species that had developed in *S. e. angulata* [2 (1936); 5 (1936); 1 (1936); 20 (1938)] and in *S. e. canadensis* [10A (1935); 10B (1935); 14 (1935); 15 (1935); 12 (1935); 11 (1935)]. All experimental snails were uninfected with larval trematodes.

Control examinations were made as follows for previously acquired natural infections with metacercariae of *C. flabelliformis* of snails from the collections used in the experiments:

- 2 (1936): 15 *L. stagnalis*, negative; 12 *S. exilis*, negative.
- 10A (1935): 15 *L. stagnalis*, negative; 12 *S. e. angulata*, negative.
- 10B (1935): 9 *S. exilis*, negative.
- 14 (1935): 6 *L. stagnalis*, negative; 16 *S. e. angulata*, negative.
- 15 (1935): *L. stagnalis*, no examination; 18 *S. e. angulata*, negative.
- 5 (1936): 2 *L. stagnalis*, negative.
- 1 (1936): 25 *S. e. angulata*, negative.
- 12 (1935): 12 *S. e. angulata*, negative.
- 11 (1935): 10 *Fossaria abrusa*, negative.
- 20 (1938): 25 juveniles of *S. e. canadensis*, negative.

## 2. Infections with *Cercariae* from *S. emarginata*

In striking contrast are the results of the experiments listed in Table 2 in which a number of snails of the varieties of *L. stagnalis* were exposed to the cercariae of *C. flabelliformis* that had developed in the varieties of *S. emarginata*.<sup>6</sup> Experiment 2 (1936) is a good example of the results of such infections. Of the 39 specimens of *L. stagnalis* examined 24 days after first exposure to infection only 14 contained any metacercariae at all, most of which had not even started to develop. In contrast, the 20 specimens of *S. exilis* in this experiment that had the same exposure to infection had an average of 124 metacercariae with more than half either "cysts" or "pre-cysts." In the other experiments in Table 2 in which specimens of *L. stagnalis* were exposed to cercariae of *C. flabelliformis* that had developed in *Stagnicola emarginata* they also showed very small infections with the metacercariae [10A (1935); 14(1935); 15(1935); 5(1936)]; and, it seems probable that part of the few metacercariae that were found had come from previous naturally acquired infections. In fact, the contrast between the results of infection of *L. stagnalis* listed in Tables 1

TABLE 3.—Records of examination of snails of the varieties of *L. stagnalis* exposed to very heavy infections with the cercariae of *C. flabelliformis* from the same host [Exp. 3A (1935)]

Snail No.	No. of days from 1st exposure to examination	Total No. of metacercariae counted	No. of "cysts"	Per cent "cysts"
1	27	1633	25	1.5
2	"	828	191	23.1
3	30	1517	714	47.1
4	"	1205	506	41.9
5	33	3092	13	0.4
6	"	2168	1918	88.0
7	"	1553	650	41.7
8	38	961	633	65.8
9	"	1263	355	28.1
10	48	1570	872	55.5
11	"	2676	947	35.4
12	56	2070	979	47.3
13	"	1543	1497	97.0
14	"	2308	2308	100.0

and 2 is very striking. In the five experiments listed in Table 2, 40 of the 68 specimens of *L. stagnalis* acquired no infection with the metacercariae of *C. flabelliformis*, while in the 8 experiments listed in Table 1, all of the 60 specimens of *L. stagnalis* acquired infections, frequently of large size, that developed normally. It seems perfectly clear, therefore, that while *L. stagnalis* is a very good second intermediate host for the cercariae of *C. flabelliformis* that develop in sporocysts in this same host, it is a distinctly abnormal second intermediate host for the cercariae of this trematode species that develop in *S. e. angulata* and *S. e. canadensis*.

### B. THE VARIETIES OF *Stagnicola emarginata* AS SECOND INTERMEDIATE HOSTS OF *C. flabelliformis*

In a large proportion of the areas in the Douglas Lake region from which our collections of *S. emarginata* were made, snails infected with the sporocysts and cercariae of *C. flabelliformis* were present, usually in low incidence, and a large

<sup>6</sup> It is interesting in this connection that in the experiments listed by Nolf and Cort in Table I of their 1933 paper, ten specimens of *L. stagnalis* in Experiment C had infections of metacercariae ranging in numbers from 402 to 1,311, although it is specifically stated that the cercariae used in infecting them came from *S. e. angulata*. It seems difficult to explain these results which are entirely different from those of all later experiments.



proportion of the snails of this species from these areas contained the metacercariae of this trematode. In collections made late in the summers they were frequently present in the juveniles as well as in the adults. Fortunately, there were a few areas where naturally acquired infections of these metacercariae were not present, from which it was possible to obtain uninfected snails for our experiments.

### 1. Infections with *Cercariae* from *S. emarginata*

*Natural infections.*—Natural infections with the metacercariae were sometimes extremely heavy in *S. emarginata* when exposed to the cercariae of *C. flabelliformis* from the same host. In the summer of 1933 it was found that specimens of *S. e. angulata* from a beach near Phragmites Flats on Douglas Lake had very large num-

TABLE 4.—Records of counts of metacercariae in natural infections in unparasitized specimens of *S. e. angulata* collected from the "Phragmites Flats Area" on July 26, 1933

Snail No.	Date of examination	Numbers of metacercariae		
		"Developing" and "pre-cysts"	"Cysts"	Total
1	8/1	688	748	1436
2	"	178	1000	1178
3	8/8	504	1057	1563
4	"	688	1033	1721
5	"	654	1360	2014
6	"	696	1263	1959
7	8/9	1130	376	1506
8	"	1112	196	1308
9	"	2011	910	2921
10	"	1172	1007	2179
11	8/14	822	633	1455
12	8/21	513	572	1085
13	"	253	1240	1493
14	"	1276	391	1667
15	8/22	862	1876	2738
16	"	50	475	525
17	"	1324	789	2113
18	"	1253	722	1975
19	"	1354	341	1695
20	"	362	455	817
21	"	504	323	827
22	"	1902	553	2455
23	"	81	204	285
24	"	332	493	825
25	"	595	462	1057
26	"	879	763	1642
27	"	8	898	906

bers of the metacercariae of *C. flabelliformis*. These metacercariae evidently came from cercariae that had developed in this same host, since the varieties of *L. stagnalis* were not found on this beach. Also, in collections of *S. e. angulata* made from this same beach during June and July, of the same year, the sporocysts and cercariae of *C. flabelliformis* had been found in 41 snails in 1,475 examined. On July 26 a collection of *S. e. angulata* was brought in from this beach. A number were immediately isolated in separate bottles for later examination. It is evident, therefore, that except for exposure to cercariae that might have escaped during the short trip across the lake from the few snails in this collection infected with *C. flabelliformis*, the metacercariae found in these snails must have been naturally acquired. Table 4 gives the counts of metacercariae from 27 snails from this collection that were not infected with larval trematodes.<sup>7</sup> It can be seen from the table that the counts were made over a period from August 1 to August 22. This delay in the examinations was due to the long time required to make counts involving such large numbers of

<sup>7</sup> The analysis of the counts of the snails of this series which were infected with larval trematodes will be given in a later paper.

metacercariae and because of the press of other work.<sup>8</sup> The counts are probably all less than the actual numbers because it is practically impossible to record all the metacercariae present in such heavily infected snails.

An analysis of the data of Table 4 gives an average of 1,531 metacercariae. While the range in numbers is considerable (285 to 2,921) it is certainly no greater than that in experimental infections. Only 6 of the snails had counts of less than 1,000. In the most heavily infected snails the metacercariae actually made up quite a large proportion of the bulk of the digestive gland, since they are localized in the hermaphroditic gland which lies embedded in this gland. Of the total number of metacercariae counted from the 27 snails listed in Table 4 only 49 per cent were encysted. Especially in the heaviest infections there were numerous small developing forms that were hardly larger than cercarial bodies. This suggested that the "crowding" in these heavy infections had caused a retardation of development. In fact, a considerable retardation is clearly shown from the counts of the 16 snails of this series which were kept in the laboratory in individual bottles without any chance of exposure to new infection from July 26 to August 21 and 22 before they were examined (cf Table 4, Nos. 12-27). This meant that the smallest developing forms, which were present in large numbers, especially in the snails with the largest metacercarial counts, had been in these snails for at least this period of almost four weeks without showing any appreciable development. In spite of their failure to develop these small metacercariae had not degenerated and were active at the time of examination.

*Experimental infections.*—The experiments in which specimens of *S. emarginata* were exposed to the cercariae of *C. flabelliformis* from this same host are listed in Table 2. It can be seen from this table that the adults of *S. emarginata angulata* in the experiments were infected with numerous metacercariae, the numbers depending on the extent of exposure to the cercariae [10A (1935); 14 (1935); 15 (1935); 1 (1936); 12 (1935); 11 (1935)]. Development appeared to be normal, although most of the experiments were terminated before development could be completed. In one experiment also [20 (1938)] juveniles were used. When examined they also contained numbers of the metacercariae that were developing normally.

From both the observations on natural infections recorded above and the results of the experiments listed in Table 2 it seems clear that *S. emarginata* both in the juvenile and adult stages is a suitable and important second intermediate host of *C. flabelliformis* when the cercariae producing the infections have developed in this same host.

## 2. Infections with Cercariae from *L. stagnalis*

To compare with the experiments of Table 2, we have the series in Table 1 in which specimens of *S. e. angulata* were exposed to cercariae of *C. flabelliformis* that had developed in *L. stagnalis*. There were six experiments in which adults of *S. e. emarginata* were used [6A (1935); 13 (1935); 5B, 5C, 5D and 5E (1937)]. In two of these experiments [6A (1935) and 5E (1937)] the numbers of metacercariae in the specimens of *S. e. angulata* were so small in comparison with the numbers in the specimens of *L. stagnalis* given the same exposure to cercariae as to suggest a host unsuitability. In contrast to these two, the other four experiments in Table 1

<sup>8</sup> The assistance of Dr. L. O. Nolf in making these counts is gratefully acknowledged.

involving adults of *S. e. angulata* [13 (1935); 5B, 5C and 5D (1937)] infected with cercariae from *L. stagnalis* showed larger numbers of metacercariae which developed about as rapidly as those in *L. stagnalis* in the same experiments. However, the numbers of metacercariae in the adults of *S. emarginata* in these experiments were very much less than in the experiments in which adults of this species were exposed to cercariae from *S. emarginata* (Table 2). These results, therefore, suggest that adults of *S. emarginata* are somewhat abnormal second intermediate hosts for the cercariae of *C. flabelliformis* that develop in *L. stagnalis*.

In addition, there were four experiments [21 (1938); 1A and 1B (1941); 2 (1941)] in which juveniles of *S. emarginata* were exposed to infection with cercariae of *C. flabelliformis* that had developed in *L. stagnalis* (Table 1). It can be seen from this table that very few metacercariae were found in any of the juvenile snails of these experiments. In experiment 21 (1938) of the 73 juveniles of *S. e. canadensis* examined 26 were negative. Of the total number of metacercariae counted from this experiment (1645) only one was encysted and 2 were in the "pre-cyst" stage. Most of the others had not even started development during the period of 36 to 37 days from infection to examination, and a number had degenerated. In Experiments 1A and 1B (1941) only 1 of the 51 juveniles of *S. e. angulata* examined contained any living metacercariae, while the specimens of *S. p. elodes* that had had the same exposure to the cercariae contained a number of metacercariae that were developing normally. Also, in experiment 2 (1941) there was no normal development of metacercariae in the juveniles of *S. e. angulata*, since in the 23 examined all the metacercariae present were very small and most of them were about the size of cercarial bodies.

These experiments indicate that juveniles of *S. emarginata* are very abnormal hosts for the cercariae of *C. flabelliformis* that develop in *L. stagnalis*. The cercariae from this host appeared to penetrate into these juveniles in considerable numbers but most of them did not even start to develop. Juveniles of this species, even of very small size, frequently harbor natural infections of the metacercariae of *C. flabelliformis*. Also, experiment 20 (1938) (cf Table 2) shows experimentally that they are suitable hosts for the metacercariae of *C. flabelliformis* when infected with cercariae that had developed in *S. emarginata*.

#### *C. Stagnicola exilis* AS A SECOND INTERMEDIATE HOST OF *C. flabelliformis*

The small number of experiments in Tables 1 and 2 involving *S. exilis* indicates that this species is a normal second intermediate host for the cercariae of *C. flabelliformis* from both *L. stagnalis* and *S. emarginata*. In the three experiments in Table 1 [3A (1935); 3B (1935); 3 (1937)] this species took infections from about 10 to 14 per cent of those obtained in groups of *L. stagnalis* that were exposed to the same numbers of cercariae from *L. stagnalis*. These very light infections were probably at least partly due to the smaller size of *S. exilis* and also to the fact that these snails tend to draw into their shells when irritated by the penetration of the cercariae. In the two experiments listed in Table 2 in which specimens of *S. exilis* were exposed to cercariae of *C. flabelliformis* from *S. emarginata* [2 (1936); 10B (1935)] the infections with metacercariae were somewhat heavier than in the experiments listed in Table 1. The time of development of the metacercariae in this host was about the same as in *L. stagnalis* (Table 1) and appeared to be more rapid than

in *S. emarginata* [cf Table 2, Exp. 10A (1935) and 10B (1935)]. Some evidence of retardation of development in *S. exilis* in heavier infections is shown by Experiment 3A (1935) (Table 1) in which there was an average of 208 metacercariae.

D. *Stagnicola palustris elodes* AS A SECOND INTERMEDIATE HOST OF *C. flabelliformis*

In Table 1 three experiments included specimens of *S. p. elodes* [4 (1937); 1A and 1B (1941)]. These snails had a moderate number of metacercariae and development appeared normal. Natural infections of the metacercariae of *C. flabelliformis*, sometimes rather heavy, were found in the area from which we obtained most of the snails of this species that we collected. This area also had numerous *L. stagnalis* and specimens of this host infected with *C. flabelliformis* were frequently found. Another series of experimental infections of this species of snail are in the experiments listed in an earlier paper (Cort, Olivier and Brackett, 1941) in which it was used for infection controls. The cercariae used in these experiments were from *L. stagnalis*. In these experiments specimens of *S. p. elodes* took infections with average numbers of metacercariae from 38 to 236. Development in *S. p. elodes* seemed to be about as rapid as in *L. stagnalis* or *S. exilis*. It, therefore, is evident that *S. p. elodes* is a normal second intermediate host for the cercariae of *C. flabelliformis* that develop in *L. stagnalis*. By an unfortunate oversight no experiments were carried out in which this species of snail was exposed to infection with the cercariae of *C. flabelliformis* that developed in *S. emarginata*.

E. *Bulinnea megasoma* AS A SECOND INTERMEDIATE HOST FOR *C. flabelliformis*

Our experiments do not give much information on how satisfactory a second intermediate host this large lymnaeid is for the cercariae of *C. flabelliformis*. We have found a few naturally acquired metacercariae in this host, usually encysted, and in one experiment of Table 1 [2 (1941)] a small infection was acquired experimentally.

F. *abrusa* AS A SECOND INTERMEDIATE HOST FOR *C. flabelliformis*

This tiny lymnaeid took infections of metacercariae when exposed to the cercariae of *C. flabelliformis* both from *L. stagnalis* and *S. emarginata* [Table 1, 4 (1935); Table 2, 11 (1935)]. The infections were light and development though normal seemed a little slower than in some of the larger species of snails. Although *Fossaria abrusa* seems to be a normal second intermediate host for *C. flabelliformis* it is so small that any individual could hardly harbor any considerable number of metacercariae without their being greatly retarded in development due to "crowding."

DISCUSSION

The most striking point that has been brought out by this study is that there is a difference in the second intermediate host relations of those cercariae of *C. flabelliformis* that develop in *L. stagnalis* and those that develop in *S. emarginata*. The cercariae from these two species of snail intermediate hosts are alike in structure and large numbers of measurements have failed to show the slightest difference in size. Also, no differences could be found in the metacercariae and adults that developed from the cercariae from these two host species. Yet when specimens of *L. stagnalis* were exposed to the cercariae of *C. flabelliformis* from *S. emarginata* al-



most no development of metacercariae occurred. Also, the cercariae of this trematode which developed in *L. stagnalis* failed almost entirely to develop in juveniles of *S. emarginata* and developed only in comparatively small numbers in adults of this species. It appears, therefore, as if within this species two host or physiological varieties have developed which utilize different species of snails for their intermediate hosts, and which differ very strikingly in their ability to utilize certain lymnaeid species as second intermediate hosts. The finding of such differences in second intermediate host relations of cercariae of the same species from different intermediate hosts suggests a variety of problems for further experimental work. Their solution, however, will be very difficult because of the complications in the life cycle of a trematode of this type which make it very hard to carry through all phases of the cycle under laboratory conditions where experiments can be adequately controlled.

The observations and experiments described above also show that the metacercariae of *C. flabelliformis* can complete their development in all the species of lymnaeid snails that we have collected in the Douglas Lake region. Great differences in the size of infections that were acquired in snails of different species were found even when they had the same exposure to the cercariae. Since snails of the different species differed so greatly in size and in their activity when exposed to infection we could not be sure that such differences were in any case due to inherent differences in susceptibility. The single experiment in which the huge lymnaeid, *B. megasoma*, was used perhaps suggests the possibility that this snail is not very susceptible to infection. Further experiments are needed, however, to test this point. Also, there appeared to be some differences in the time taken for the metacercariae to complete their development in the different species of hosts. Here again, the experiments are not sufficiently controlled in relation to temperature and numbers of metacercariae to draw the conclusion that there are inherent differences in this respect.

Several of the experiments showed clearly that the size of the infection has a great influence on the speed of development. In snails infected with large numbers of metacercariae there was a very marked retardation in development. In fact, in the series of *S. e. angulata* that had natural infections ranging from 1,000 to 3,000 metacercariae it appeared as if the development of some of the metacercariae was completely inhibited. It is suggested that this retardation of development in heavily infected snails may be due simply to "crowding" which produces severe competition for the available space and food supply. However, the possibility cannot be excluded that some sort of specific immunity is acquired by the host as a reaction against repeated infections with the metacercariae which may finally retard or inhibit the development of those from later infections. The close contact of the metacercariae with the tissues of the host during the whole period of their development might certainly be expected to produce some reaction. Perhaps both factors have a part in the retardation of development in heavy infections.

#### SUMMARY

The varieties of *Lymnaea stagnalis* and *Stagnicola emarginata* in the Douglas Lake region of Northern Michigan are common intermediate hosts for the development of the cercariae of the duck strigeid, *Cotylurus flabelliformis*. The cercariae of this species that develop in *L. stagnalis* and those that develop in *S. emarginata* appear to represent two different physiological or host varieties of this species, since they

differ in their second intermediate host relations. The variety, the cercariae of which develop in *S. emarginata*, fail almost entirely to develop into metacercariae in *L. stagnalis* while they utilize very effectively *S. emarginata* as a second intermediate host. On the other hand, the cercariae of the variety that develops in *L. stagnalis* utilize this same species very effectively as a second intermediate host, but do not develop so well in adults of *S. emarginata*, and hardly at all in juveniles. A number of other lymnaeid snails also serve as second intermediate hosts for *C. flabelliformis*. They belong to four different genera and differ greatly in size and habits. Differences in the size of the infections with the metacercariae acquired by the snails of the different species were found even when they had the same exposure to infection. These differences could be explained, at least in part, by differences in size and activity of the different snail species. It was found that in very heavy infections with metacercariae there was a marked retardation in development, perhaps due to limited space and food supply.

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STUDIES ON *DIENTAMOEBEA FRAGILIS* (PROTOZOA). IV.  
FURTHER OBSERVATIONS, WITH AN OUTLINE OF  
PRESENT-DAY KNOWLEDGE OF THIS SPECIES

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I. INTRODUCTION

Since *Dientamoeba fragilis* was first described as a new genus and species of intestinal amoeba from man by Jepps and Dobell in 1918 a number of different writers have published observations and interpretations concerning it. The most comprehensive paper is that of Dobell (1940), who redescribed the organism, gave an account of nuclear and cell division, recorded observations on cultures and on transmission experiments, and pointed out similarities between *Dientamoeba* and *Histomonas*.

In several previous publications (1936, 1937, 1939, 1944) I have discussed variations in the morphology of the animal, its life history, and its possible pathogenicity. It is the purpose of the present communication to record additional observations combined with an outline of our present knowledge of this interesting amoeba.

During the past 20 years over 100 cases of infection with *D. fragilis* have been encountered in the diagnostic work of my laboratory. In each case, fixed and stained slides have been made from the feces. Most of the critical studies have been made from these slides.

For assistance in obtaining material I am indebted to many persons, but more especially to Dr. A. D. Waltz and Dr. John H. Arnett. Dr. Sarah H. Stabler has rendered helpful technical assistance. In part, these studies have been aided by grants from the Special Research Fund of the University of Pennsylvania and from the Penrose Fund of the American Philosophical Society.

II. PROBLEMS OF DIAGNOSIS

The frequent absence of *Dientamoeba* from lists of parasites in survey reports or its mention with a very low percentage of incidence, may indicate either that the methods employed were unsuitable for its recognition or that the investigators were not sufficiently familiar with it to identify it. When the methods are applicable to cysts alone, this species will naturally not be seen. Hood (1940) noted that in a laboratory in Chicago, *D. fragilis* had not been recorded for more than 2000 fecal examinations. After a summer at the Gorgas Memorial Hospital in Panama, where experience was gained in the identification of *Dientamoeba*, an incidence of 4.2% was obtained from 164 individuals examined during the succeeding 10 months—an incidence slightly higher than that for *Entamoeba histolytica*.

For the diagnosis of *Dientamoeba*, the following are needed: 1) sufficient experience in its recognition; 2) soft or fluid feces; 3) examination while the feces are still fresh; 4) recognition of the animals in fresh smears and/or on properly fixed and stained slides. Cultures may reveal positives missed by direct examination methods.

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In feces diluted with salt solution, specimens of *D. fragilis* are spherical in shape with granular or finely alveolate protoplasm. The nuclei are not visible, but food inclusions may be. After a time rather broad, clear, thin pseudopodia may be extended, usually from only one side. In fresh smears treated with Iodine solution, the nuclei remain invisible, but the granular character of the cytoplasm persists. If fresh feces are diluted with water, these amoebae swell up and burst, releasing the endoplasm, after which the ectoplasmic shell remains as a hollow sphere (Hakansson, 1935). While this behavior appears to be characteristic I should not wish to depend upon it alone for diagnoses.

The most reliable method is to make properly fixed and stained slides. Smears of feces are commonly fixed in Schaudinn's fluid and stained with iron-haematoxylin. In such preparations, however, the nuclei often fail to stain properly. If from 15 to 20% of glacial acetic acid is added to Schaudinn's fluid, just before using, the nuclei of *Dientamoeba* generally stain much more distinctly (Johnson, 1935; Wenrich, 1937, 1941). If the usual mixture with 5% of acetic is diluted with an equal amount of distilled water, the results are often quite satisfactory. Modified Bouin's fluid (picro-formol-acetic in the proportions 75:15:10) fixes *D. fragilis* very well. It is a good routine practice to fix some smears in Schaudinn's and some in modified Bouin's. Iron-haematoxylin is the stain of choice. Haemalum stains the nuclei very well and they react positively to the Feulgen technique. Some of the many variations from the typical nuclear structure may be disconcerting, especially in the over-sized individuals with single atypical nuclei (cf. Figs. 13, 28).

It is well to prepare fixed and stained slides of all positive or doubtful cases. In our survey of 1060 students (Wenrich, Stabler and Arnett, 1935), 61.3% of the *Dientamoeba* infections were recognized on stained slides alone; and in the survey of food handlers (Wenrich and Arnett, 1942), 36.4% were detected on stained slides alone.

Culture methods may add greatly to the number of positives recognized. Many persons have recorded successful cultures of this species, for example: Cleveland and Collier (1930), Svensson (1935), Brug (1936a) and Dobell (1940). These papers should be consulted for details.

### III. MORPHOLOGY AND LIFE HISTORY

1. *Size.* According to Jepps and Dobell (1918), the diameters range from 3.5 to 12  $\mu$  and most authors have repeated these figures, which are undoubtedly typical. However, both the range and the average size may vary from one population to another. In previous papers (1936, 1937) I called attention to diameters up to 17-18  $\mu$ , and Fig. 13 illustrates an individual 18 $\times$ 22  $\mu$ . These larger animals are commonly mononucleate (Fig. 13) but may be multinucleate (Fig. 10). Figs. 11 and 12 show individuals 4  $\mu$  in diameter, with 1 and 2 nuclei. Animals as small as 3  $\mu$  in diameter are sometimes seen on slides from feces. For the case described in 1937, measurements made on amoeba in 2 successive stools gave an average diameter for the first stool of 8.8  $\mu$ , somewhat less than for the second (9.36  $\mu$ ). Hakansson (1936) noted variations associated with the condition of the host. When diarrhea was severe, the average diameter was greater (11  $\mu$ ), than when symptoms abated. In his cultures, Dobell (1940) noted the occurrence of "giants," up to 20  $\mu$  and "dwarfs," as small as 3  $\mu$  in diameter. As noted elsewhere (Wenrich, 1944) larger



size may be correlated with polyploidy. The nucleus of the large animal shown in Fig. 9 is apparently octoploid.

2. *Cytoplasmic structure.* In living examples of *Dientamoeba*, the cytoplasm may appear to be granular or frothy. In stained specimens it often appears to be finely or more coarsely vacuolated; but there are many variations from the typical alveolar condition. Larger vacuoles may be few (Fig. 4) or many (Fig. 20). Occasionally there is one large central vacuole (Fig. 29). Extensive vacuolation represents a degenerate condition. Homogeneity is another. This may affect patches here and there (Figs. 9, 10, 28), the endoplasm alone (Fig. 26), or the entire cytoplasm (Figs. 11, 17, 25). Figs. 23 and 24 apparently represent intermediate conditions.

In stained specimens there may or may not be evident a line of demarcation between ectosarc and endosarc. The degree to which this character finds expression varies with individuals, with populations, and doubtless varies with the conditions of the environment at the time the animals were fixed. In some cases the ectosarc is very sharply demarcated (Figs. 13, 26, 27, 30, 39). The relative width varies greatly; in Figs. 26 and 39 it is narrow, wider in Figs. 27 and 30. In these last two figures the zone of contact between the ectosarc and endosarc is deeply stained. In Fig. 31 a similar narrow, deeply-stained zone surrounds a small area of cytoplasm containing one nucleus. In another example (not illustrated) each nucleus was similarly enclosed in an island of cytoplasm marked off by a deeply stained boundary. In Fig. 30 the ectosarc has a peculiar radial organization. The deeply staining boundary layer probably plays an important role in the activities of the animal.

The food inclusions seen on prepared slides are diverse, as indicated by the few examples illustrated in Figs. 32-40. Various types of bacteria are found, including chains (Fig. 32) and filaments (Fig. 36); also yeasts (Fig. 33), *Blastocystis* (Figs. 35, 40), starch grains (Fig. 34), and unidentifiable parts of cells (Figs. 37, 38). Cannibalism sometimes occurs (Fig. 39), and the parasite, *Sphaerita*, is not uncommon (Fig. 40). There seems to be less hypertrophy in cells of *Dientamoeba* parasitized with *Sphaerita* than in some other endozoic amoebae. Dobell (1940) noted the avidity of these organisms for starch grains in culture and also that they ingested red blood corpuscles.

The methods of ingestion appear to be similar to those described for *Entamoeba muris* and *E. ranarum* (Wenrich, 1941a). Clusters of bacteria may be taken in by broad food cups (Fig. 41), while a rod or a filament appears to be ingested through a narrow opening which may (Fig. 43) or may not (Figs. 42, 44) be accompanied by a deeply stained "pharynx."

In my study of *Histomonas* from wild ring-neck pheasants (1943), tube-like extensions of the cytoplasm were found in about 15% of the animals on stained slides. Fig. 49 illustrates one of these. It will be seen that the walls of this extension are made up of the layer of cytoplasm that lies between the ectosarc and the endosarc. Somewhat comparable structures have been found in *Dientamoeba* (Figs. 45-48). They may be broad (Figs. 46, 47), apparently containing food elements, or may be relatively narrow (Figs. 45, 48). These differentiations may have extensive internal components, as shown in Figs. 45 and 46. They are much more common in some populations than in others.

3. *Nuclear number.* *Dientamoeba* is characterized by the presence of 2 nuclei

in a great majority of the individuals in most cases; but the percentage varies greatly in different populations. Jepps and Dobell (1918) recorded an average of about 80% of binucleates and this is apparently typical. But Kudo (1926) reported only 12.2% of binucleate amoebae in the case that he studied. Different stools from the same host give different percentages. In the case of the child suffering from ill health described in the second of these studies (1937), the binucleates in 5 different stools were only 73, 38, 9, 46 and 57%. Since many of the mononucleates, when they are in the majority, are larger than usual, there may be a correlation between monoclearity and symptoms, as well as between size and symptoms (cf. Hakansson, 1936).

Various authors have reported the occasional presence of more than 2 nuclei in an individual (cf. Dobell, 1940). Many populations in my collection of slides seem to lack such supernucleates, while in others they are not uncommon. Figure 10 represents an individual with 7 nuclei, one larger than the others; on the same slide, one other with 7, one with 5 and about a dozen with 3 or 4 nuclei were observed.

4. *Nuclear size.* Nuclear size tends to be correlated with cell size but there are many exceptions in some populations. Most nuclei range from 0.8 to 3  $\mu$  in diameter, but in oversized animals, they may be larger. In figure 13 the nucleus is 4  $\mu$  and in figure 20 it is 5  $\mu$  in diameter. These are, of course, abnormal specimens.

5. *Nuclear structure.* According to the original describers, the structure of the nuclei is vesicular, there being a central chromatic area called the "karyosome," composed of a number of small chromatin granules embedded in a weakly staining matrix; surrounding the "karyosome" there is a clear area enclosing which is the delicate nuclear membrane with little or no chromatin on its inner surface. A few fine fibers radiate out from the "karyosome" to the nuclear membrane and small granules may be seen at the point of contact with the membrane.

Most other authors have agreed to this description but some have noted considerable deviations from it (cf. Wenyon, 1926, and my previous papers). On the basis of material from cultures, Dobell (1940) has revised the description, stating, among other things, that neither matrix nor radiating fibers are seen. Kudo (1926) and others have verified the existence of the radiating strands and elsewhere (1944) I have interpreted them as chromosomal fibers, comparable to those reported by Cleveland et al (1934) for certain hypermastigote flagellates. Apparently culture amoebae do not show some details of structure as clearly as do those from feces.

In my series of papers on this species I have called attention to the great amount of variation in the appearance of the nuclei as observed on fixed and stained slides. To the variations previously described, Dobell (1940) has added a "true 'resting' condition" in which there is a prominent endosome with the remainder of the chromatin dispersed through the nuclear space and up against the nuclear membrane (cf. Fig. 4). The formation of such a "resting" condition would, theoretically, be a normal process, but I have found this stage mostly in populations showing abundant evidence of degeneration, and failed to find it in an apparently normal population in which many individuals were undergoing nuclear division. Therefore, it seems doubtful that it is a necessary part of the nuclear division cycle.

Post-divisional changes in the nucleus appear to be of two general sorts: A) those which take place within the central area (= the original "karyosome" of Jepps and Dobell); and B) those during which the chromatin spreads out through the

nuclear space to the membrane to produce the "resting" condition. Under the first category (A) may be noted several types of changes: 1) the characteristic condition is for one or more of the 4 telophase chromosomes to divide once to produce chromomeres which become somewhat diffuse in appearance. One of these chromosomes may fail to divide, retaining its staining capacity to become the endosome (Figs. 14, 39). This latter may also divide, in which case the endosomes may be very difficult to identify (Figs. 24, 31); 2) successive divisions may give rise to more than 8 chromomeres or "granules" (Figs. 1, 17; diploidy is an alternative interpretation here); 3) 3 of the 4 telophase chromosomes may become more diffuse without dividing while the fourth remains as a more deeply stained endosome (Figs. 23, 26, 27, 38, etc.); 4) the "granules" may lose their identity in a diffuse mass (Figs. 15, 16); 5) the chromatin may become resolved into a reticulum (Figs. 2, 13).

Dispersion out to the nuclear membrane (category B, above) may apparently take place in different ways. As previously shown (1944) dispersal may be radial from the central cluster of granules to the nuclear membrane; succeeding this, or possibly as an alternative method, a reticulum is formed with an endosome remaining (Fig. 3). In either case, eventual dispersion may be fairly complete and either uniform (Fig. 36) or with a well-defined layer against the membrane (Fig. 4). It is possible that some of the conditions listed under A, above, might become transformed into this final stage of the "resting" condition, but the evidence indicates a difference between categories A and B.

When dispersion takes place according to one or the other of the first 4 types in category A, the endosome appears to be derived from one of the 4 telophase chromosomes. This would be in harmony with the now generally accepted idea that at least one type of nucleolus in metazoan cells is derived from special loci in particular chromosomes. Chen (1936) showed definite relationships between nucleoli and certain chromosomes in another protozoön, *Zelleriella intermedia*. In the types of dispersion mentioned in category B, the evidence points to the origin of the endosome from more than one, possibly all the chromosomes, as seems to be the case for the "chromocenter" in certain kinds of metazoan nuclei. Apparently the 2 types of endosomes (nucleoli) do not appear in the same nucleus in *Dientamoeba*, but our information on this point is incomplete.

Dobell (1940) mentions individuals with 2 endosomes in ... nucleus. In one population I found a considerable number of these (Fig. 5) and even one with 4 endosomes. I have interpreted the additional endosomes as evidence of diploidy and tetraploidy. Fig. 9 shows an oversized individual containing a hypertrophied nucleus with 8 endosomes, presumably a case of octoploidy.

Some of the more extreme types of dispersion mentioned above may represent degeneration. In one population nearly all the nuclei, in both binucleates and mononucleates were in a condition similar to that in Fig. 15, probably all degenerate. Another kind of nuclear degeneration appears in the form of pycnosis. Figs. 6, 7 and 8 are from a slide on which most of the members of the population are normal, but some show the pycnotic nuclear condition illustrated in Figs. 7 and 8. Fig. 6 presents an intermediate condition. In Fig. 25 is depicted an apparently degenerating polyploid nucleus in a state of arrested division. It is, of course, impractical to illustrate and describe all the different degenerative conditions.

The kind of animals that survive longest in feces should be a matter of interest.

Figs. 14–20 illustrate individuals from feces kept at room temperature for 24 hours, and Figs. 21 and 22, specimens from 48-hour-old feces. One could argue that they must be fairly normal to be able to live that long, or, conversely, that they must be abnormal after having lived that long in a supposedly unfavorable environment. For the most part they appear to be abnormal, but the 48-hour survivor represented by Fig. 22 appears to be fairly normal. The nuclei in Figs. 15 and 16 are doubtless abnormal. In Fig. 18 one nucleus is fairly normal while the other is hypertrophied and abnormal. The nucleus in Fig. 19 shows 4 prophase chromosomes, probably not normal. That in Fig. 20 contains 8 “granules,” 6 of which are seen to be double; the other 2 may also be double but not oriented so as to reveal their true condition, or they might be endosomes. This is probably a diploid animal with a hypertrophied nucleus and cell body.

6. *Nuclear division.* For *Dientamoeba*, nuclear division has been incompletely described by Kudo (1926), Brug (1936), Dobell (1940) and Wenrich (1939, 1944). Relatively few details are provided by Kudo and Brug and the fuller accounts by Dobell and Wenrich are admittedly incomplete. Dobell's description is based upon culture material while mine are based on slides from feces. Differences in our interpretations may be due, in considerable measure, to this difference in source.

Nuclear division is mitotic. Dobell (1940) believes that the chromosome number is 6, but I have shown that it is 4 (1939, 1944). There is one smallest, one largest, and 2 of intermediate size. They show a longitudinal split at mid-prophase. Dobell states that the desmose is extranuclear. I first described it as intranuclear (1939) but later discovered a centriole on the nuclear membrane surrounded by a small astral area. This divides to spin out the desmose which comes to lie near the center of the spindle in late prophases, metaphases and early anaphases. It may possibly fit into a fold in the nuclear membrane, as in certain flagellates (cf. Cleveland et al, 1934). In late anaphases it takes a more peripheral position and, after nuclear constriction, persists as a strand connecting the daughter nuclei for a relatively long time, usually until plasmotomy occurs. Unbalanced anaphases, seen on some slides, offer a clue to the inequality of nuclear size observed in occasional individuals.

7. *Cytoplasmic divisions.* Dobell (1940) described cytoplasmic division in living animals from his cultures. The cell elongates and constricts into 2 approximately equal parts, each containing one nucleus. Both Dobell and I find that animals undergoing division usually display a persisting desmose, as revealed by stained slides. I have records of only 2 individuals without desmoses which were elongated and constricted, so that apparently such animals seldom divide. At present there is no evidence that polynuclear animals with 3, 4 or more nuclei are capable of cytoplasmic division.

8. *Viability in feces.* Aside from its multiplication by binary fission the life history of *D. fragilis* is very incompletely known. Jepps and Dobell (1918) in their original description noted its rapid disappearance from feces but stated that degenerate individuals with large central vacuoles (cf. Fig. 29) might live in that condition for 2 or 3 days at room temperature. Temperature does affect survival as is indicated by the following observations. In Feb., 1927, a stool containing large numbers of *Dientamoeba* was obtained within 3 minutes after deposition. A portion was placed in a covered dish on the ledge outside the window where the temperature



was 3.5° C. At the end of 2 hours practically all the animals had disappeared, while in the portion kept at room temperature there was no noticeable decline in numbers, and 23 hours later there were still some to be found. In the first of these studies (1936) I called attention to the survival of considerable numbers in feces kept for 24 hours at summer room temperature (August, 1935) (cf. Figs. 14-20), and some few in 48-hour-old feces (Figs. 21, 22). Hakansson (1936) noted survival at room temperature for 48 hours in stools with much starchy residue, and for 3 or 4 days in saline smears sealed to the slides with vaseline. It is obvious that *D. fragilis* does not always disappear from stools as quickly as earlier reported.

Mononucleate animals seem to survive longer in feces than binucleates. On slides made at the time of the original examination in the case mentioned above (August, 1935), 22% of the animals were binucleate. On slides made 24 hours later only 9% showed 2 nuclei. This would suggest that binucleate animals were disappearing faster than the mononucleates, though division of some of the binucleates might have occurred. On one of the earlier slides, measurements of 100 individuals gave a range in diameter from 5 to 12  $\mu$  with an average of 7.9  $\mu$ . Only one was as much as 12  $\mu$  in diameter. On a slide from the 24-hour-old feces measurement of 100 animals gave a range of diameters from 5 to 15  $\mu$  with an average of 9.07  $\mu$ . Eight specimens were 12  $\mu$  or more in diameter (cf. Figs. 14-20). The average diameter of the binucleates was 10  $\mu$  indicating that they had shared in the size increase. Similar results were obtained from another stool from the same host when kept at room temperature for 24 hours. These observations suggest that there was an actual increase in size during the 24-hour period.

9. *Absence of cysts.* Nearly all students of *Dientamoeba* have recorded failure to find cysts. Kofoed (1923) reported cysts but an examination of his figures indicates that he was observing animals with thin clear ectosarcs (cf. Fig. 26). In the first of these studies (1936) I suggested that small deeply-stained individuals (cf. Figs. 11, 17, 23-25) might be "pseudocysts," having in mind the rounded-up and cyst-like stages of some species of *Trichomonas*. It seems more likely, however, that these represent one form of degeneration. They appear to be no more common in 24-hour old feces (Figs. 14-20) than in fresher material.

#### IV. RELATIONS TO HOST

1. *Incidence.* As noted by Dobell (1940) and others the geographical distribution of *D. fragilis* is world-wide. Its small size, absence of cysts, rapid disappearance from feces and difficulty of recognition readily account for the delay in its description until 1918 (Jepps and Dobell). It is somewhat surprising and disappointing that, since the publication of the original description, reports of so many surveys fail to record its presence or report only a very small percentage of infection. A review of 19 surveys made in the United States, during the past decade, in Washington, D. C., Georgia, Florida, Louisiana, Mississippi, Kentucky, Indiana, Missouri and California, indicates that 65,253 persons were examined without recording the presence of *Dientamoeba* in any of them. Seven other surveys, made in the same decade, in So. Carolina, Kentucky and California, record the examination of 19,001 persons with an average incidence of 0.64%, the highest recorded being 3% in 678 children in a Los Angeles hospital (Kessel and Sinitsin, 1938).

In Philadelphia, Pa., Wenrich, Stabler and Arnett (1935) found an incidence

of 4.3% in 1060 students, when only one normally passed stool per person was examined. Employing the same methods, Wenrich and Arnett (1942) found 3.9% in 190 food handlers, but as a result of more than one examination for some of these individuals, an incidence of 7.4% was obtained. Stabler (1941) reported 2.8% in 106 students taking his parasitology course (one examination per person). Hinshaw and Showers (1934) found 0.3% in 368 patients at the University of Pennsylvania hospital; while Rothman and Epstein (1941) reported cases in their private practice they gave no percentage, but Rothman and Laskey (1943) found 2.3% in 306 employees of a large hospital. There is no reason to suppose that *Dientamoeba* is more prevalent in Philadelphia than in other similar communities.

In certain other studies, incidences recorded have been relatively high. Svensson (1928) found 8.7% in formed stools from 46 asylum patients, but the incidence was increased to 23.9% when purged stools were examined. Andrews (1934) in his study of the relative advantages of normal vs. purged stools found 3 cases in 50 persons (6%). Cleveland and Collier (1930) discovered 6 infections in 100 persons when culture methods were employed. Brug (1936a) reported 12% incidence in 80 asylum patients by direct examination methods but increased this to 36% by the use of cultures. Hood (1943) obtained incidences of 4.8% and 7.5% for 1126 hospital patients and 265 hospital employees, respectively, examined at a Chicago hospital from 1938 to 1942 during which years iron-haematoxylin smears were made in addition to other routine methods. For *Entamoeba histolytica* the incidences were only 3.46% and 1.88% for the same groups. When properly trained observers employ appropriate techniques, the incidence of *Dientamoeba* will be found to be much higher than present survey results indicate.

A high incidence of infection with *D. fragilis* has been reported for the inmates of certain institutions for mental patients. For example, more than 50% in an asylum in Sweden (Svensson and Linders, 1934); 36% in an asylum in Holland (Brug, 1936a); and 42% in an asylum in Panama (Hakansson, 1937). On the other hand, Young and Ham (1941) reported only 1% in 142 mental patients in South Carolina and Burrows (1943) only 0.1% in 1418 patients at the same institution. Kmecza (1939) failed to find any *Dientamoeba* in 1200 patients in a hospital in Indiana as did Hopp (1944) in 771 patients in another hospital in the same state. The experience of Hood (1940, 1943) in Chicago, would suggest that *Dientamoeba* is to be found in that section of the country if care is taken to identify it.

2. *Possible pathogenicity.* Evidence that *Dientamoeba* may sometimes be a pathogenic agent has been presented from time to time, for example by Gittings and Waltz (1927), Hakansson, (1936), Wenrich (1937), Saper (1939) and Hood (1940). It may possibly be significant that of the 8 original cases listed by Jepps and Dobell (1918), 6 were soldiers with a history of dysentery or other disturbance of the digestive tract. Another was a native of Britain who had never been abroad, but *Dientamoeba* was found when he was suffering from an attack of diarrhea attributed to a chill. The eighth case was the one in whose feces Wenyon had observed this amoeba. Gittings and Waltz reported 2 cases of children with poor health and a high percentage of eosinophiles accompanied by infection with *D. fragilis*. Hakansson reported an infection in an adult in Panama where the presence of large numbers of *Dientamoeba* was associated with aggravated diarrhea and other gastro-intestinal symptoms. Both the symptoms and *Dientamoeba* disappeared with carbarsone treatment.

Wenrich (1937) gave a more complete history of "case 1," of Gittings and Waltz, which later developed liver abscess. Unfortunately no microscopic examination was made of the exudate from the abscess when it was drained, and the patient moved away making further study impossible. With Dr. Waltz a number of other children with poor health and infection with *D. fragilis* were studied. Some, but not all, also had high eosinophilia.

Sapero (1939), in a report on non-dysenteric amoebiasis among 450 U. S. Navy men, recorded that of 106 who harbored *Entamoeba histolytica*, 43.4% had complaints; of 108 who had no intestinal Protozoa, only 7.4% indicated symptoms; and of 236 who had other intestinal Protozoa, 16.1% had complaints. Hosts of *Entamoeba coli*, *Endolimax nana* and *Iodamoeba bütschlii* showed no more symptoms than those without Protozoa. On the other hand, complaints were recorded for 27.3% of 44 cases of infection with *Dientamoeba*, "A finding which suggests a pathogenic rôle for this organism."

Hood (1940) found 7 cases of *D. fragilis* infection, 3 children and 4 adults, among 164 persons examined. One of the children, in whom *E. histolytica* was also found, had definite intestinal disturbances. Another child had diarrhea and 11% of eosinophiles. The other child's mother reported that it had had worms. The 4 adults had diarrhea and other intestinal symptoms. One patient left the hospital before treatment was instituted, but for the other 3, treatment removed both the amoebae and the symptoms. Wenrich, Stabler and Arnett (1935) found that the students who harbored *Dientamoeba* reported somewhat more gastro-intestinal disturbances than those who were infected with *E. histolytica*.

I have had 4 periods of infection with *D. fragilis* lasting 5 months, 2 years, 2 years and 2 months, and 4 months, respectively. There was a 2-year interval between the first and second periods and between the second and third, and a 7-year interval between the third and fourth periods. *Dientamoeba* has not appeared during the past 7 years. For the first period, *D. fragilis* was associated with *Endolimax nana* throughout and with *Chilomastix* for a short time. During the second and third periods no other protozoön was found. For the fourth period, *E. nana* and *Enteromonas* were also present and still persist. At the beginning of each period, *Dientamoeba* was discovered when a stool was examined because of an attack of diarrhea. In each infection the diarrhea gradually abated but there followed a period of increased fatigability and low resistance to colds. No treatment was ever taken for the infections and the organisms disappeared spontaneously.

There is, of course, no definite proof of pathogenicity in all this "evidence," but the suggestion that this amoeba can play a pathogenic rôle in some persons is very strong. Since most of the hosts of *Entamoeba histolytica*, at least in temperate climates, are carriers showing no very obvious symptoms, it is probable that a still larger percentage of hosts of *Dientamoeba* are also carriers without symptoms. At present there is no evidence that it invades tissues but it appears to be associated with more generalized evidences of ill health suggesting that the effects are toxic in nature. More studies will be needed to determine whether or not *D. fragilis* can be the cause of illness, but an effort to settle the question should be made.

3. *Transmission.* The mode of transmission of *D. fragilis* from host to host is still unknown. There is evidence that, in more temperate climates, at least, it has an incidence as high as, or higher than, that for *Entamoeba histolytica*. It shares

with other parasites an unusually high incidence among patients in mental asylums. The familial factor may be operative since Hakansson (1937) reported infections in 4 members of a Panamanian family of 9, and in all members of a white family of 4. One might therefore assume that this amoeba would be transmitted by contamination of food and drink as are other intestinal Protozoa; but of this we have little knowledge. Dobell (1940) swallowed a culture of *Dientamoeba* but remained negative. Equally negative were the results of his attempts to infect two monkeys by feeding them cultures and by injecting one per anum. Perhaps these culture amoebae were low in invasive capacity. Dobell also tried to infect 9 young chicks per anum and obtained a temporary (7-day) infection in only one of them.

Infections in other animals have been reported. Hegner and Chu (1930) found *Dientamoeba* in 2 out of 44 wild monkeys (*Macacus philippinensis*) examined in the Philippines, and Knowles and Das Gupta (1936) recorded an infection in another monkey (*Silenus irus*) examined in India. Regandanz (1936) reported its persistence for 15 days in one of 4 rats injected rectally with human feces containing *D. fragilis*. I fed *Dientamoeba* in milk to a rat but got no infection.

Hakansson's (1935) observations that these amoebae swell and burst when placed in water indicates that they are not transmitted by contaminated water. At various times I have placed feces containing *D. fragilis* in boiled pond water or in boiled hay infusion, but got neither survival nor cysts. The absence of cysts makes the problem of transmission a very difficult one.

Dobell (1940) believes that *Dientamoeba* is closely related to *Histomonas*, which may be transmitted by the eggs of the caecal worm, *Heterakis gallinae*. He suggests that *D. fragilis* might be similarly transmitted, possibly by *Trichuris trichiura* or by *Ascaris lumbricoides*. Experiments are needed to test this hypothesis.

#### V. COMPARISON BETWEEN *Dientamoeba* AND *Histomonas*

Dobell (1940) suggests that *D. fragilis* has many of the characteristics of a flagellate. "*Dientamoeba* is, indeed, a typical flagellate except for the important circumstance that it possesses no flagella." He cites similarities to *Histomonas meleagridis*, as follows: *Histomonas* grew in all the media that he used for *Dientamoeba*; it ingests red blood corpuscles in cultures, as does the latter; some of the nuclei contain a group of chromatin granules reminiscent of those of *Dientamoeba*, while others have a "resting" stage like that of the latter species; during division the desmose is a conspicuous feature and may persist until cell division in both species; Tyzzer's (1934) figures (Plate IV) of living *Histomonas*, except for the flagella on some individuals, could pass as those of *D. fragilis*.

To these similarities may be added from the present study, the formation of the peculiar tube-like cytoplasmic differentiations on both organisms, and associated with this the (often) deeply stainable boundary zone between the ectosarc and endosarc. Another flagellate character is the presence of chromosomal fibers in interphase and prophase nuclei.

However, since *Dientamoeba* has never been known to develop flagella, it must still be classed as an amoeba. On the other hand, it is sufficiently distinct from other amoebae to be placed in a family by itself; but I doubt if any useful purpose would be served by introducing a new family name into the literature at this time.



## VI. SUMMARY

1. Over 100 cases of infection with *D. fragilis* have appeared in diagnostic work of my laboratory. Fixed and stained slides have been made in each case. Most of the critical observations have been made on these slides.

2. For diagnosis, training in recognition is required. Fresh soft feces should be examined. Cultures are useful in detecting positives. Properly fixed and stained slides should be made for all apparently positive or doubtful cases. From 15 to 20 percent of glacial acetic acid added to Schaudinn's fluid increases the stainability of the nuclei. Picro-formol-acetic in the proportions of 75:15:10, fixes this species very well. Iron-haematoxylin is best for staining.

3. Diameters range from 3 to 20  $\mu$ ; most being from 5 to 10  $\mu$ . Larger individuals are usually mononucleate but may be multinucleate. Nuclear structure in larger mononucleates is commonly atypical making identification difficult.

4. The cytoplasm is typically finely alveolate or granular in appearance. The ectoplasm may be demarcated from the endoplasm by a deeply stainable zone, which is probably related to important activities of the cell. In degenerate animals one to many large vacuoles may be seen. Degenerate cytoplasm may become homogeneous in patches, in the endoplasm alone, or in the entire cell.

5. Food bodies include bacteria of many kinds, yeasts, *Blastocystis*, cellular fragments and starch grains. Ingestion of red blood cells has been reported. Cannibalism may occur and parasitization by *Sphaerita* is common.

6. Tube-like extensions of the cytoplasm, comparable to those reported for *Histomonas*, occur also in *Dientamoeba*.

7. Although most populations have about 80 per cent of binucleate individuals, there is much variation; in one population only 9 per cent had two nuclei. Super-nucleate animals with 3 and 4 nuclei occurred occasionally in some populations; others with 5 to 7 nuclei appeared less frequently.

8. Nuclei of normal individuals vary from 0.8 to 3  $\mu$  in diameter. Large mononucleates may have nuclei 4 or 5  $\mu$  in diameter. Variations in nuclear structure are notable. Typically there is a central area containing 4 to 8 "granules" or chromatic lumps. One is usually more heavily stained, being designated the endosome. Dispersive processes are varied and may lead to a "resting" condition with a distinct endosome and finely divided chromatin out to and against the nuclear membrane. Among nuclei of this type two endosomes are not uncommon in some populations; 4 and 8 endosomes have been seen. These are interpreted as representing diploid, tetraploid and octoploid conditions. Extreme forms of dispersion may be degenerate. Nuclear pycnosis is another degenerative condition.

9. During nuclear division 4 chromosomes divide on a spindle which includes a desmose stretched between polar centrioles. In the early prophase the centriole has a small astral area around it. In midprophase the chromosomes show a longitudinal split. After nuclear constriction the desmose persists for a relatively long time, commonly until cytoplasmic division takes place.

10. Although these amoebae disappear rapidly in feces, some may survive 48 hours or more at summer room temperature. At 3.5° C they disappeared in 2 hours. Survivors are more commonly uninucleate and larger than usual.

11. Most authors are agreed on the absence of cysts for this species.

12. When properly trained observers employ appropriate methods, the incidence of *Dientamoeba* as revealed in surveys may be as great as, or greater than, that of *Entamoeba histolytica*. Several observers have reported unusually high incidences in the inmates of mental asylums.

13. Evidence is accumulating that *Dientamoeba* can be a disease producing agent in some instances. More studies are needed to determine clearly its possible pathogenic potentialities.

14. The mode of transmission is as yet unknown. Dobell (1940) was unable to infect himself or two monkeys with culture amoebae. He suggests that it may be transmitted by the eggs of some nematode worm, as is true of *Histomonas meleagridis*.

15. Dobell (1940) called attention to the similarities between *D. fragilis* and *Histomonas meleagridis*, such as general appearance, nuclear structure and the persistence of the desmose. Additional similarities recorded here are: 1) the deeply-staining zone between the ectosarc and endosarc, and 2) the formation of tube-like extensions from and within this zone. Another flagellate character is the presence of chromosomal fibers in the interphase and prophase nuclei. However, since *Dientamoeba* has never been observed to produce flagella, it must still be classed as an amoeba. On the other hand, it is so different from other amoebae that it might well be placed in a separate family.

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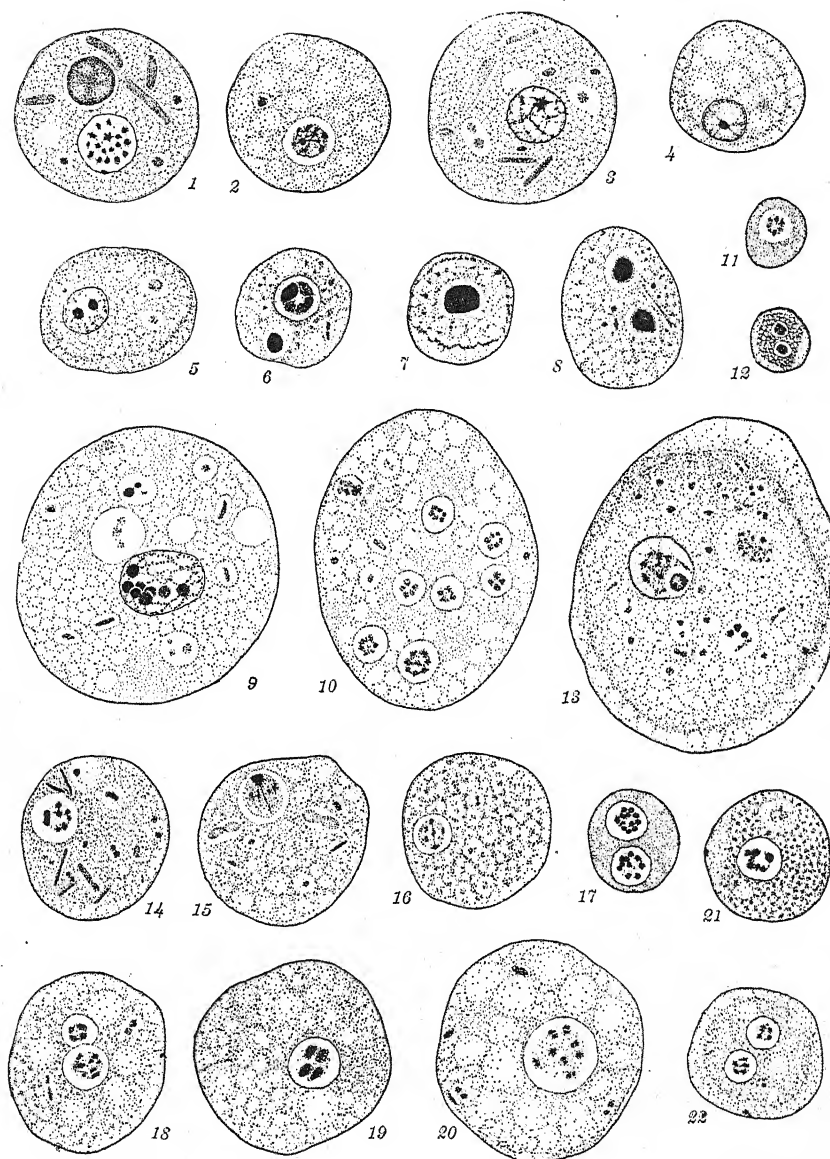
## VIII. EXPLANATION OF PLATES AND FIGURES

All figures have been drawn with the aid of a camera lucida at an initial magnification of X3000 and have been reduced about 1/3 in printing. All are from prepared slides which had been fixed in Schaudinn's fluid, with 5 to 20 per cent of acetic acid added, or with picro-formal-acetic in the proportions of 75:15:10. All were stained with iron-alum haematoxylin.

## PLATE I, p. 336

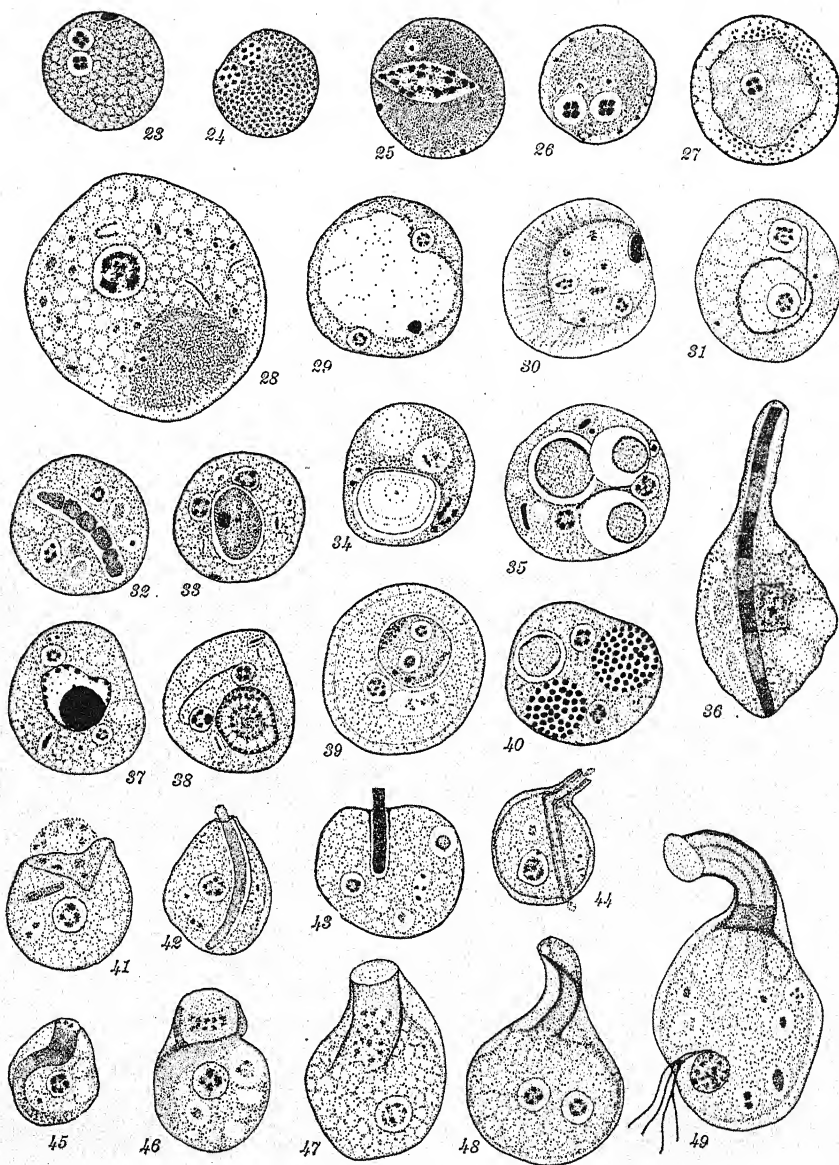
- FIG. 1. Individual with about 16 "granules," besides the endosome, in the central nuclear area.
- FIG. 2. A reticulum has formed in the central nuclear area.
- FIG. 3. The chromatin radiates out from the endosome and forms a reticulum which reaches to the nuclear membrane.
- FIG. 4. Nucleus in the "resting" condition.
- FIG. 5. Nucleus has dispersed chromatin and 2 endosomes.
- FIG. 6. Early stage in nuclear pycnosis.
- FIGS. 7 and 8. Mononucleate and binucleate examples with pycnotic nuclei.
- FIG. 9. Oversized individual with a large nucleus containing 8 endosomes.
- FIG. 10. Large individual with 7 nuclei, one larger than the others.
- FIG. 11. Small mononucleate with homogeneous cytoplasm, 4  $\mu$  in diam.
- FIG. 12. Normal-looking binucleate, 4  $\mu$  in diam.
- FIG. 13. "Giant," 22  $\times$  18  $\mu$ , with nucleus 4  $\mu$  in diam.
- FIGS. 14-20. Specimens from feces kept 24 hrs. at summer room temperature.
- FIG. 14. Nucleus has 6 "granules" besides the double endosome.
- FIG. 15. Chromatin diffuse in the central nuclear area; 1 endosome.
- FIG. 16. Chromatin diffuse in central area; 2 endosomes.
- FIG. 17. Animal with homogeneous cytoplasm and 2 nuclei, each with more than 8 "granules."
- FIG. 18. Animal with 2 nuclei different in size.
- FIG. 19. Nucleus contains 4 prophase chromosomes.
- FIG. 20. Nucleus, 5  $\mu$  in diam., contains 8 "granules"; probably diploid.
- FIGS. 21 and 22. From feces kept at summer room temperature for 48 hrs.
- FIG. 21. Animal with degenerate-looking nucleus and cytoplasm.
- FIG. 22. Animal with fairly normal nuclei and cytoplasm.





## PLATE II, p. 338

- FIG. 23. Nuclei look normal; cytoplasm approaching homogeneity.  
FIG. 24. Nuclei look degenerate; cytoplasm approaching homogeneity.  
FIG. 25. Cytoplasm homogeneous; nucleus, apparently polyploid, in a state of arrested division.  
FIG. 26. Endoplasm homogeneous; ectoplasm distinct; nuclei look normal.  
FIG. 27. Sharply defined boundary between ectosarc and endosarc, each of which has distinctive structure.  
FIG. 28. Large mononucleate with large patch of degenerate cytoplasm.  
FIG. 29. Cytoplasm has large central vacuole.  
FIG. 30. Ectosarc thick, with radial organization.  
FIG. 31. Deeply staining "boundary layer" surrounds small area of cytoplasm containing one nucleus.  
FIGS. 32-39. Animals with various types of food inclusions.  
FIG. 32. A bacterial chain besides other food bodies.  
FIG. 33. Contains a yeast cell.  
FIG. 34. Contains 2 starch grains.  
FIG. 35. Contains 3 specimens of *Blastocystis*.  
FIG. 36. Animal has ingested a long filament.  
FIGS. 37 and 38. Contain unidentifiable parts of cells.  
FIG. 39. Cannibalism; a large one has ingested a smaller one.  
FIG. 40. Contains 2 specimens of the parasites, *Sphaerita*; 1 of *Blastocystis*.  
FIG. 41. Large food cup partially enclosing cluster of bacteria.  
FIG. 42. Long rod, mostly ingested.  
FIG. 43. Rod being ingested; note deeply-stained "pharynx."  
FIG. 44. Ingested rod or filament.  
FIG. 45. "Cytoplasmic differentiation"; cf. fig. 49.  
FIG. 46. Combination of "cytoplasmic differentiation" and food cup.  
FIG. 47. Partly extended tube-like "differentiation."  
FIG. 48. Projecting narrow "differentiation."  
FIG. 49. Example of *Histomonas* from ring-neck pheasant, showing tube-like "differentiation" from layer between ectosarc and endosarc.









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## THE TROMBICULID MITES (CHIGGER MITES) AND THEIR RELATION TO DISEASE<sup>1</sup>

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The subject of this address, "The Trombiculid Mites and Their Relation to Disease," is one in which the speaker has long been interested as an investigator. It is believed to be timely, and can with propriety be regarded as a part of the symposium on "Parasitology in Relation to the War" which is held here this morning.

Under the name of chiggers, or redbugs, the larvae of our common trombiculid mite are doubtless familiar to most of our members who live in the Southern States. Here they long have been known as an annoyance to man because their attacks cause a skin eruption and intense itching; but, if this were the only kind of injury caused we would have no great interest in them as wartime pests. It is now known, however, that in several parts of eastern Asia and the islands of the Pacific other similar species transmit a disease frequently fatal to man.

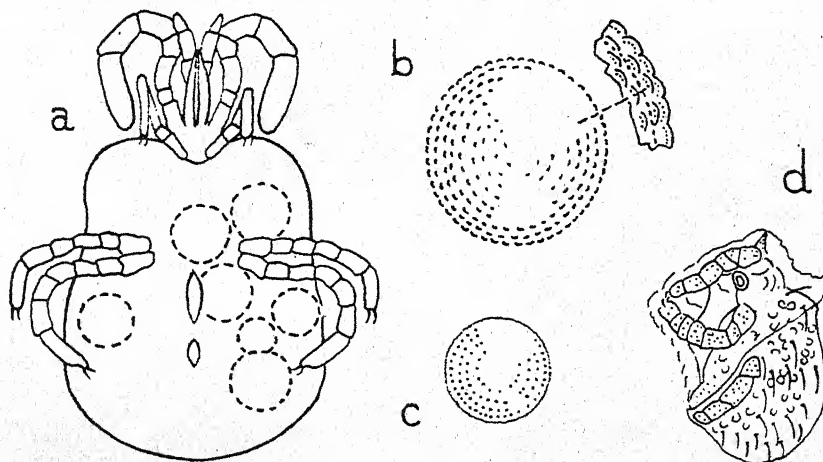


FIG. 1. a, female of *Trombicula akamushi*, showing more than one fully formed egg,  $\times 40$  (after Kawamura and Ikeda); b, egg (with detail of chorion) that was forced through a rupture of abdomen of *Eutrombicula goldi*; c, egg of *E. alfreddugèsi* fully formed in the abdomen of female; d, larva of *T. akamushi* yet enclosed in cuticular exuviae of embryo, but after casting its chorion, or eggshell,  $\times 90$  (after Miyajima and Okumura). Figures b and c,  $\times 100$ . (Original.)

Received for publication, October 26, 1944.

<sup>1</sup> Address of the retiring President, American Society of Parasitologists, September 12, 1944, Cleveland, Ohio, being also a part of the symposium on "Parasitology in Relation to the World War."

## LIFE HISTORIES

The chigger, or larval stage of a trombiculid mite, is the only one that is parasitic and is the only one usually detected in nature by the general entomologist or parasitologist. This is because the other stages—the nymph, adult, and egg—are concealed in the soil.

The eggs from which the larvae hatch usually are produced one at a time in the body of the female, but according to Kawamura and Ikeda (1936) in the common

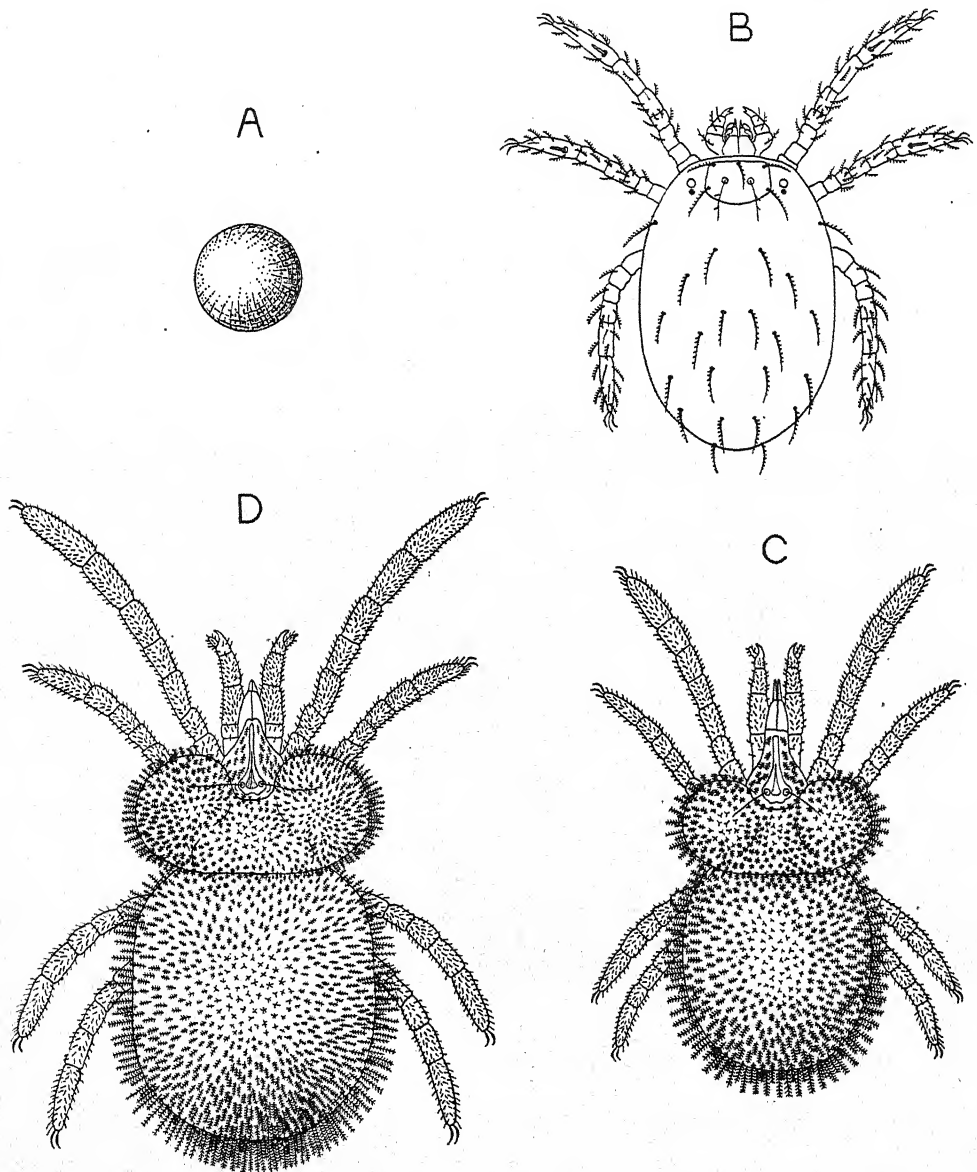


FIG. 2. The different stages in the life cycle of *Eutrombicula alfreddugèsi*; A, fully developed egg as found inside of abdomen of female,  $\times 100$ ; B, larva reared from isolated female,  $\times 100$ ; C, a nymph reared from a larva on a young toad, *Bufo* species,  $\times 70$ ; D, adult reared from a larva on a box turtle, *Terrapene carolina*,  $\times 60$ . (Original.)

Japanese trombiculid, *Trombicula akamushi* (Brumpt), more than one egg (Fig. 1, a) may reach full development at a time. The egg (Fig. 1) of the different species are very minute and spherical but may have a thick chorion.

Miyajima and Okumura (1917) have described a deutoval stage as occurring in the life cycle of *T. akamushi*. However, what they observed (Fig. 1 d) is really nothing more than the cast cuticular exuvia of the fully developed embryonic larva. This is observed in many of the more generalized insects and is not a true deutovum, as is found, for example, in the spiders and certain other arthropods.

The trombiculid larva (Fig. 2, B) is very different in its structure from the succeeding stages. It is very small, seldom being more than two-fifths of a millimeter in length, has only six legs, and the chelicerae possess recurved, hooklike processes or teeth. The body bears a conspicuous dorsal plate and is sparsely clothed with setae.

In the best studied trombiculid mite, *Trombicula akamushi*, a quiescent stage is known to intervene between the larva and the active nymph. At least Miyajima and Okumura (1917) have figured such a stage which is known as the nymphochrysalis. Apparently this stage is homologous with the protonymph of many other kinds of mites. In the formation of the nymphochrysalis the appendages and some of the inner structures of the body undergo histolysis. These appendages are reformed not inside the appendicular shells of the cast larval skin but instead under the cuticle of the body. Also, the appendages of the nymphochrysalis are not distinctly segmented, and they, as well as the body proper, are devoid of setae. The nymphochrysalis is inactive and always is found inside the old larval skin.

Active nymphs (Fig. 2, C) of several species of trombiculids have now been reared both in the New and in the Old World. They differ greatly from the larvae in being much larger, and in having four pairs of legs. In addition the body has numerous setae, and instead of the dorsal plate, a dorsal crista and area sensilligera (Fig. 3) are present. Morphologically nymphs differ from adults in the following three respects: They are smaller; each has but two pairs of genital suckers instead of the three present in the adult; and each palpal tibia has only two subterminal spines (Fig. 3), instead of the three or four found in the adult.

The adults resemble the nymphs very closely not only in structure but also in habits and the two sexes are remarkably alike. In fact, but few investigators have ever mentioned the sexual differences. However, if the genital area of a well-cleared and stained specimen is examined, certain chitinized structures are clearly revealed (Fig. 4) that indicate the sex.

Concerning the numerical ratios of the two sexes but little is known. Among the adults of *Eutrombicula alfreddugèsi* in the United States National Museum collection, which came from various localities in the United States, 25 individuals could be clearly determined as to sex. Of these, 13 are males and 12 females.

#### OUR PRESENT KNOWLEDGE CONCERNING THE LIFE CYCLE OF *Eutrombicula alfreddugèsi* (OUDEMANS)

This occasion is probably an opportune one for summarizing what is known on the life cycle of our common North American chigger, *Eutrombicula alfreddugèsi* (Oudemans). The first published report on this matter was in Science (59 (1515): Sup. p. XIV) in 1924 and contained a statement that I had found the adult of our common chigger. This referred to results which were published early the following



year (Ewing, 1925a) and in which the following facts were presented: Adult females, collected from places where there was a known infestation of *E. alfreddugèsi*, were isolated in individual breeding cells and kept alive from several to many days. From two such females several larvae of *E. alfreddugèsi* were obtained.

The nymphs of *E. alfreddugèsi* probably were first reared by Miller (1925a and 1925b). In some very important experiments, in which for the first time snakes

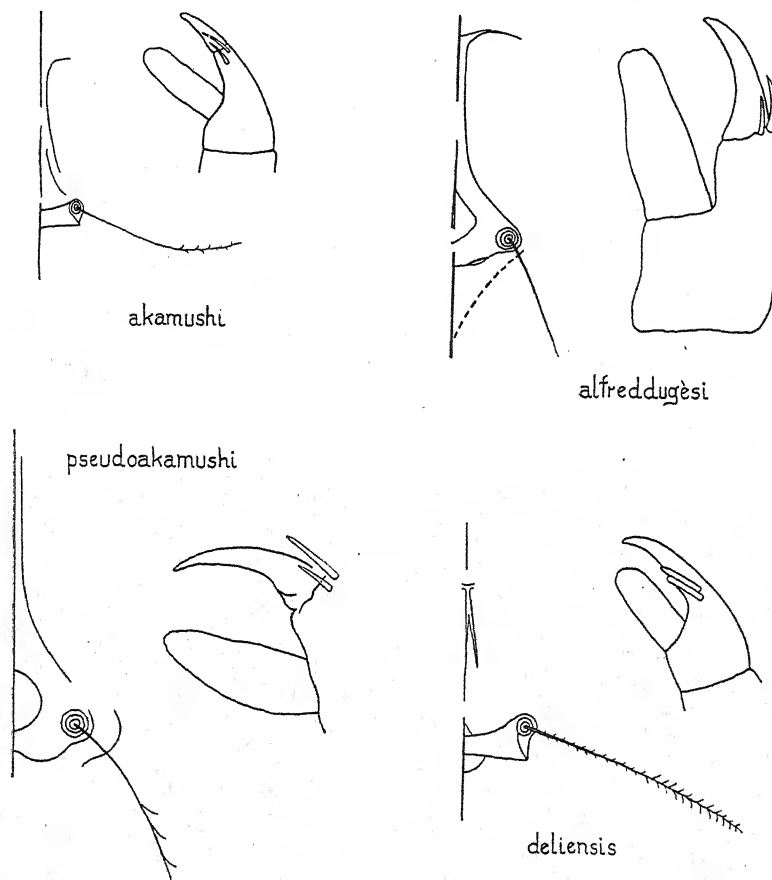


FIG. 3. Drawings (greatly but not equally enlarged) of the crista and area sensilligera (right half of each), and the distal portion of the palpus (dorsomedian views) of nymphs of four species of trombiculids. Those of *Trombicula akamushi* (Brumpt) are after Miyajima and Okumura, 1917; of *T. deliensis* Walch, after Walch, 1924; of *Eutrombicula pseudoakamushi* (Hatori), original from a specimen reared by Walch; of *E. alfreddugèsi* (Oudemans), original from specimens reared by the writer.

were shown to be natural hosts of our common chigger, he reared nonparasitic individuals. These he regarded as adults, claiming that the nymphal stage was absent. While this conclusion was evidently in error, Miller's experiments were of much importance since for the first time a nonparasitic form of an American trombiculid was reared from the parasitic larvae.

The year following, the writer (Ewing, 1926a) reared many nymphs and two adults of *E. alfreddugèsi* from live infested box turtles, *Terrapene carolina* (Linnaeus), taken at North Beach, Md. It was found at that time that "the chiggers

engorge rapidly on the box-turtle and drop off without difficulty." On the other hand, on snake hosts the chiggers had been found to engorge very slowly and to detach themselves with difficulty.

Since rearing the nymphs and adults from our common box turtle, I have reared nymphs alone from young toads, *Bufo* species. These rearings were as follows: On three occasions infested live young toads were collected at Great Falls, Va., during the months of July and August. From a total of 48 young toads thus taken 5 active nymphs were reared.

It will be noted that in these various rearing experiments it was easy to obtain nymphs from engorged larvae which had dropped from the host individuals, but

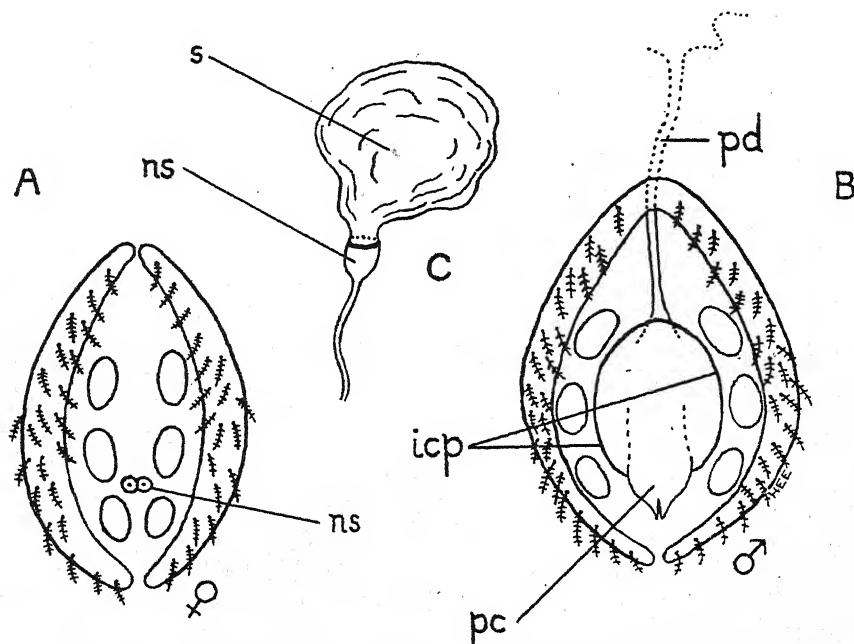


FIG. 4. Structures about the genital openings of adults of *Eutrombicula alfreddugèsi* (Oudemans); A, of female (inner genital plates not shown); B, of male (inner genital plates not shown); C, detail of a (?) sacculus of female. Lettering as follows: *icp*, internal chitinous plates lining genital opening; *ns*, neck of (?) sacculus (in A, deeply stained in natural position from below; in C, seen in profile); *pc*, penial cone; *pd*, duct of penis; *s*, (?) sacculus. All greatly enlarged. (Original.)

from the large number of larvae obtained by the writer from more than 50 individual hosts (box turtles and young toads combined) only 2 adults were reared. These results are typical of those obtained by other workers; in all cases considerable difficulty was experienced in obtaining adults.

Although particular efforts were made in some of the rearing experiments to induce females of *E. alfreddugèsi* to deposit eggs, none was obtained. In fact, I have never seen the egg of our common chigger mite except in the body of the female.

Now to summarize, the instars involved in the life cycle of our common North American trombiculid as known at present are the undeposited egg, the larva, the active nymph, and the adult. There remains to be found the deposited egg (if eggs are in reality deposited) and the nymphochrysalis (if such a stage really exists).

## CLASSIFICATION

The classification of the trombiculid mites can be regarded as beginning with the establishment of the oldest included genus, *Trombicula* Berlese (1905). When Berlese established this genus, and for several years thereafter, it was not known that the adults, upon which it was based, were the parents of chiggers. In his review of the adult forms of the whole family TROMBIDIIDAE, Berlese (1912) divided *Trombicula* into two subgenera, which together included six species. In the same year Oudemans (1912) published his summary of what was known of the larvae of the two families TROMBIDIIDAE and ERYTHRAEIDAE. It is in this comprehensive work

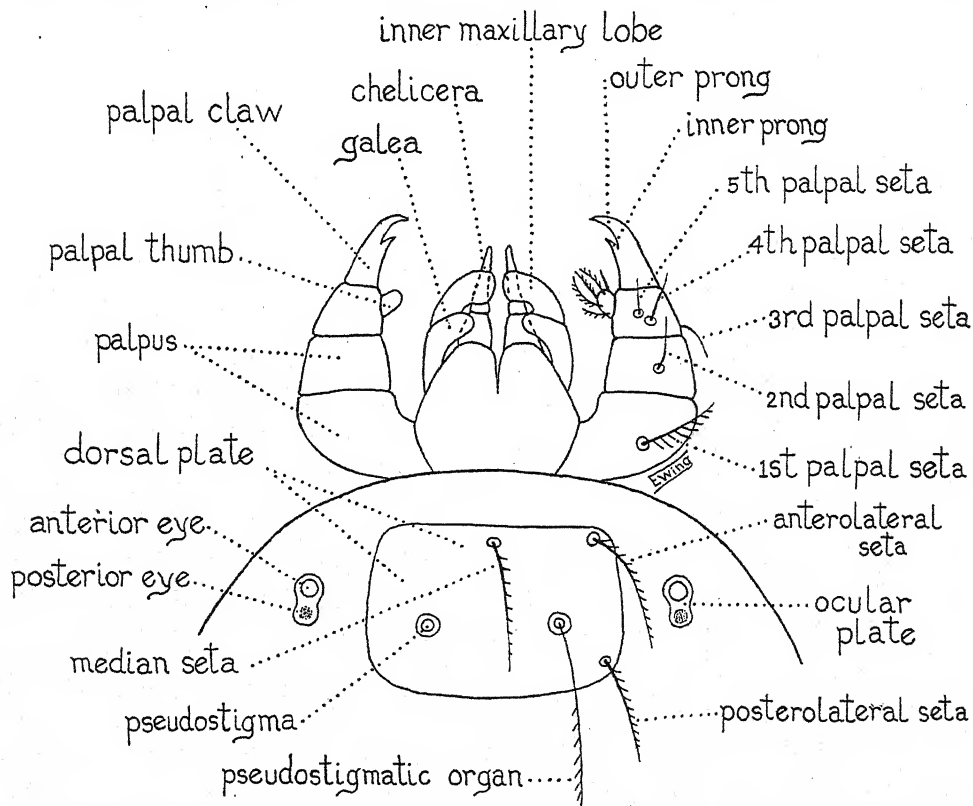


FIG. 5. Drawing of anterior part of larva of *Eutrombicula göldii* (Oudemans), dorsal view, with setae omitted on left side. (Greatly enlarged and with structures labeled.) (Original.)

that the trombiculid larvae receive their first extensive treatment, yet, strange to say, the name TROMBICULA is not mentioned in its pages. The time had not yet arrived for the proper association of the larval forms with the adults.

When the subfamily TROMBICULINAE was established (Ewing, 1929b) and its six genera keyed out, several species had been reared to the nymphal stage and a few to the adult. Later this key was expanded (Ewing, 1938b), in the form of a classification, to include 15 genera.

Womersley and Heaslip (1943) in their extensive paper on the trombiculid mites of the Austro-Malayan and Oriental Regions recognize 19 genera of the TROMBICULINAE, including the genus *Myotrombicula*, which was described as new. This genus was established for a very unusual species in which the chelicerae and palpi are said

to be modified for hair clasping. Although the genus apparently belongs to the TROMBICULIDAE, I have been unable to place it properly in the key to genera here given.

Recently (Ewing, 1944) the subfamily TROMBICULINAE has been raised to the rank of a family and a new subfamily created for the divergent genus *Hemitrombicula* Ewing. These changes are noted in the revised classification that follows.

*A Revised Classification of the Larvae of the Trombiculid Mites, or TROMBICULIDAE*

- A. Each tarsus with 2 unequal claws; pseudostigmatic organs represented by a pair of simple setae; setae on both body and appendages, simple. Known only from North America ..... *HEMITROMBICULINAE* Ewing  
Contains but a single genus ..... *Hemitrombicula* Ewing
- AA. Each tarsus 3-clawed, outer claws nearly always equal, forming a pair, middle claw usually longer than others; pseudostigmatic organs, when simple, long and more or less flagelliform ..... *TROMBICULINAE* Ewing
  - B. Body constricted near its middle; abdomen with a posterodorsal plate. Eggs laid attached to hairs of host. Known only from New Guinea and Australia ..... *Guntherana* Womersley
  - BB. Body not constricted near its middle; abdomen without plates. Eggs laid, as far as known, unattached to host. Known from most of the warmer regions of the world.
    - C. Middle tarsal claw stouter than paired claws; eyes absent  
..... *Riedlinia* Oudemans
    - CC. Middle tarsal claw more slender than paired claws; eyes usually present.
      - D. Dorsal plate of cephalothorax with a single median seta on or near its anterior margin.
      - E. Pseudostigmatic organs strongly clavate or capitate.
      - F. Anterolateral setae of dorsal plate large, barbed, similar to posterolateral setae.
      - G. Each chelicera with a row of dorsal teeth; palpal claw usually with 2 prongs  
..... *Schöngastia* Oudemans
      - GG. Each chelicera with only a single dorsal tooth.
      - H. Palpal claw undivided, or with 2-3 prongs.
        - I. Dorsal plate with a distinct crest in front of pseudostigmata and its posterior half with circular striations  
..... *Paraschöngastia* Womersley
        - II. Without these characters  
..... *Neoschöngastia* Ewing
      - HH. Palpal claw with 5-7 prongs, the accessory prongs being in 2 or 3 pairs; dorsal tooth of chelicerae vestigial or absent  
..... *Euschöngastia* Ewing
    - FF. Anterolateral setae of dorsal plate minute, simple. Parasitic on bats ..... *Doloisia* Oudemans



EE. Pseudostigmatic organs setiform or flagelliform, and usually with barbs or lateral branches.

F. Each chelicera with a row of dorsal teeth; ventral tooth sometimes absent.

G. More than 3 subequal dorsal teeth on each chelicera; dorsal plate well developed; median seta barbed . . . . . *Odontacarus* Ewing

GG. Three unequal dorsal teeth on each chelicera; dorsal plate vestigial; median seta simple. Living under the surface of the skin of amphibians

*Endotrombicula* Ewing

FF. Each chelicera with a single dorsal tooth and usually with a ventral tooth.

G. Palpal claw simple; dorsal setae less than 22; pseudostigmata much nearer anterior margin of dorsal plate than posterior . . . *Crotiscus* Ewing

GG. Palpal claw divided into 2 or 3 prongs; dorsal setae 22 or more; pseudostigmata usually about equal distance between anterior and posterior margins of dorsal plate or nearer posterior margin.

H. Palpal claw typically divided into 3 prongs; dorsal abdominal setae usually more than 30 . . . . . *Trombicula* Berlese

HH. Palpal claw divided into 2 prongs, which usually are in the same horizontal plane in mounted specimens.

I. Setae on dorsal plate, exclusive of pseudostigmatic organs, 9

*Heaslippia* Ewing

II. Setae on dorsal plate, exclusive of pseudostigmatic organs, 5.

J. Anterolateral setae of dorsal plate setiform, similar to posterolaterals.

K. Dorsal abdominal setae 22 (counting posterior marginal pair) and in unengorged larva with the following arrangement, 2-6-6-4-2-2; ventral body setae 14, 1 pair being postanal

*Eutrombicula* Ewing

KK. Dorsal abdominal setae over 22 (counting posterior marginal pair); ventral body setae more than 14,

more than 1 pair being  
postanal

*Acariscus* Ewing

JJ. Anterolateral setae of dorsal  
plate short, very stout, peglike

*Fonsecia* Radford

DD. Dorsal plate of cephalothorax without a median seta on or near  
its anterior margin but sometimes with a pair of submedian  
setae.

E. Dorsal plate with a pair of submedian setae on or near  
anterior margin.

F. Dorsal plate with an anterior median process but  
without crista.

G. Posterolateral setae present on dorsal plate.

H. Posterolateral setae of dorsal plate not  
swollen or clavate, but similar in form to  
anterolateral setae; dorsum of abdomen  
thickly beset with setae not situated on  
tubercles.

I. Chelicera with but a single tooth on  
upper margin; either first or second  
palpal seta barbed

*Comatacarus* Ewing

II. Chelicera with a row of teeth on up-  
per margin; both first and second  
palpal setae simple

*Acomatacarus* Ewing

HH. Posterolateral setae clavate, thus differ-  
ing in form from anterolateral setae; dor-  
sum of abdomen sparsely clothed with  
stout setae situated on tubercles

*Leeuwenhoekia* Oudemans

GG. Posterolateral setae absent from dorsal plate

*Apolonia* Torres and Braga

FF. Dorsal plate without anterior median process but  
frequently with crista; each chelicera obliquely flat-  
tened at distal end, forming a "spearhead" with teeth  
on its lateral margin.

G. Dorsal plate subrectangular, with only simple  
setae; submedian setae situated on an allantoid  
thickening; outer tarsal claws but slightly, if  
any, stouter than middle claw

*Whartonia* Ewing

GG. Dorsal plate not subrectangular, with barbed  
or branched setae; submedian setae not situated  
on any thickening; outer tarsal claws usually  
much stouter than middle claw

*Hannemania* Oudemans

EE. Dorsal plate without a pair of submedian setae.

F. All setae on dorsal plate (exclusive of pseudostigmatic organs) marginal.

G. Dorsal plate with 4 or more pairs of lateral setae ..... *Gahrlepiea* Oudemans

GG. Dorsal plate with less than 4 pairs of lateral setae.

H. Dorsal plate with 3 pairs of lateral setae; eyes present ..... *Schöngastiella* Hirst

HH. Dorsal plate with 2 pairs of lateral setae; eyes absent ..... *Walchia* Ewing

FF. Some of setae on dorsal plate not marginal; eyes usually present ..... *Gateria* Ewing

#### DISTRIBUTION

Trombiculid mites are found in greatest abundance and diversity in areas with a hot, moist climate and an abundance of vertebrate life. They are reported from all the zoogeographical regions of the world and parasitize, in their larval stage, representatives of four of the five classes of vertebrates, the amphibians, the reptiles, the birds, and the mammals. In the tropics they extend vertically to the tops of many mountains, yet in the arctic regions they appear to be unknown. The distribution of each of the four best known species is here considered in some detail.

#### *The Distribution of the Common North American Chigger Mite, Eutrombicula alfreddugèsi (Oudemans)*

*Eutrombicula alfreddugèsi* has an almost continuous distribution, except for certain areas made artificial by man, over all the Southern States except for the northwestern part of Texas, and in addition over the Coastal Plain and Piedmont districts of the North Atlantic States and the southern half of the Upper Austral life zone of Merriam, in the Mississippi Valley. Outside this area it occurs in many areas (Fig. 6, dotted) where there exist widely scattered local "ecological islands" in which conditions are particularly favorable for it.

Some significant records indicating the limits of distribution of *Eutrombicula alfreddugèsi* (Oudemans): The following records of *E. alfreddugèsi*, except for the 2 from Mexico, are here published for the first time. The specimens upon which they are based have all been identified by the writer, except those from Mexico which were identified by Islas (1943).

*Arizona*: Winslow, on hen, August 27, 1919. *Bahama Islands*: On "lizard," July 25, 1930. *California*: Coachella, on road runner, *Geococcyx californianus*, October 12, 1931, and Nice, on "common brown lizard," November 7, 1939. *Cuba*: Cabo Cruz, on black and white warbler, *Mniotilta varia*, August 31, 1930. *Delaware*: Newark, on meadow mouse, *Microtus pennsylvanicus*, August 10, 1939. *Haiti*: San Michel, on "lizard," March 23, 1925. *Illinois*: Yorkville, on "pheasant," July 19, 1941. *Kansas*: Winfield, on "collared lizard," July 27, 1942. *Mexico*: Puebla State; Matamoros and Acatlan, on man (reported by Islas, 1943). *Michigan*: Camp Bryan, on pilot black snake, *Elaphe obsoleta*, July 1931. *Minnesota*: Minneapolis, on spreading adder, *Heterodon contortrix*, July 1922. *Ohio*: Nile

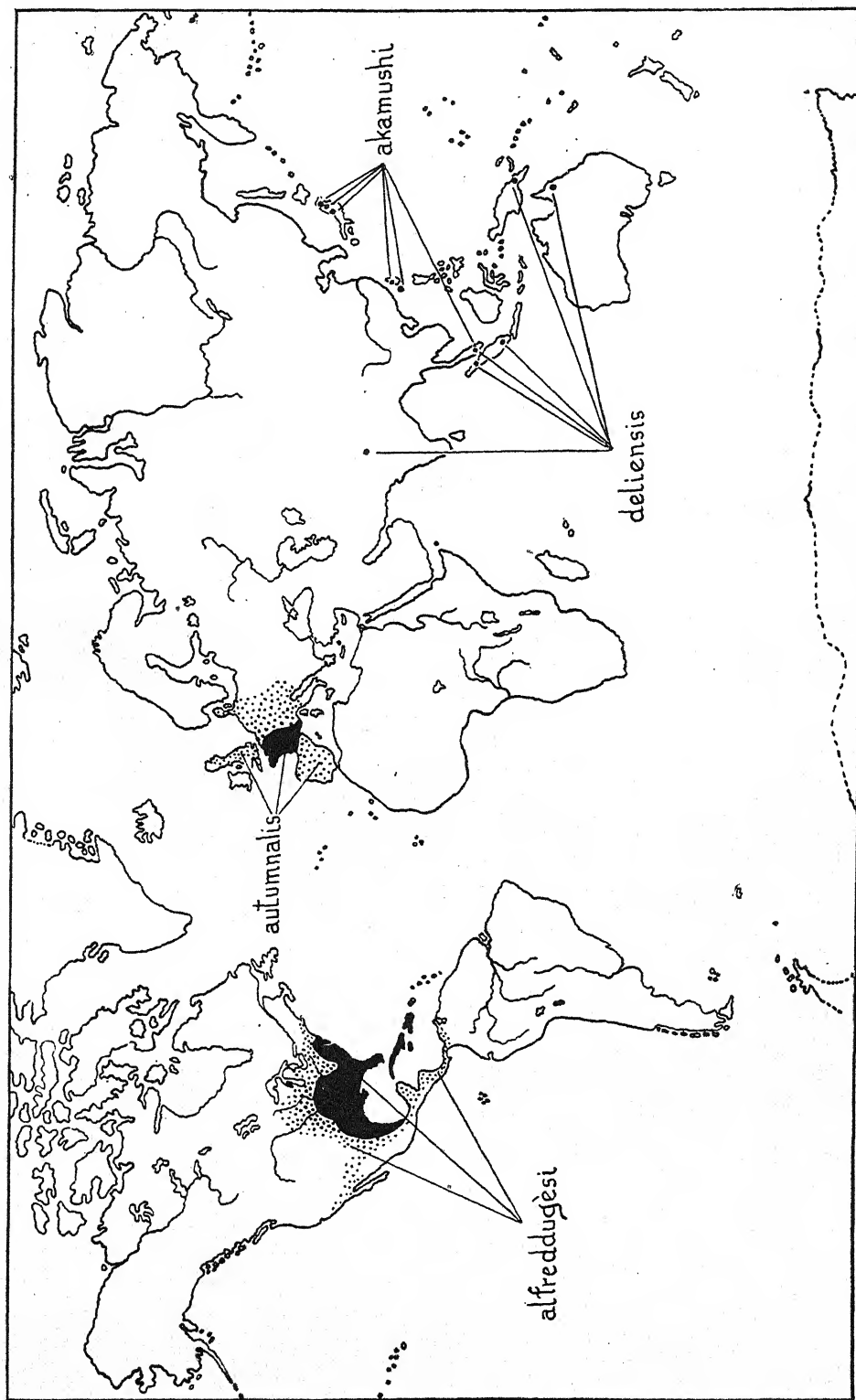


FIG. 6. The geographical distribution of four species of trombiculid mites, *Eutrombicula alfreddugèsi* (Oudemans), *Trombicula autumnalis* (Shaw), *T. akamushi* (Brumpt), and *T. deliensis* Walch. Black indicates approximate area of maximum or near maximum abundance; stippling, the approximate area with scattered "ecological islands" of mites. (Original.)



township, on rabbit, *Sylvilagus floridanus*, July 29, 1937. Panama: Aguas Buenas, on a snake, *Spilotes pullatus*, March 31, 1931; Gatun Lake, Canal Zone, on eastern green heron, *Butorides virescens virescens*, June 5, 1931; Parito, on coati, *Nasua narica panamensis*, July 8, 1931. Pennsylvania: Stone Valley, adults (3 males and 1 female), June 1923. Texas: Uvalde, on "horned toads," May 4, 1943. Venezuela: Chama River, on lizard, *Anadia bitaeniata*, date (?).

#### *The Geographical Distribution of Trombicula autumnalis (Shaw)*

André (1928a, 1930a) has summarized the distribution records for *Trombicula autumnalis* (Shaw). Some of these records, however, are based upon cases of trombiculiasis (= trombidiasis) and for that reason are not entirely reliable. This is particularly true for the records from south central Europe where trombiculiasis is sometimes caused by another species.

According to André, *Trombicula autumnalis* is widely distributed in western and central Europe. Most of André's records are for France and western Germany, and probably central, western, and southern France is the region most severely infested by this species. In England and Scotland the mite occurs sparingly. The same appears to be true of Spain and Italy.

Some significant records of the occurrence of *Trombicula autumnalis* (Shaw): The following are a few of the more significant records as mentioned by André (1930): Denmark: Jutland. England: Isle of Wight, Lincolnshire, Surrey, Wiltshire, Yorkshire. Germany: Meiningen (Valley of the Saale), Teplitz (Bohemia). Scotland: Berwick, East Lothian. Spain: Locality not mentioned.

#### *The Distribution of Trombicula akamushi (Brumpt)*

Notwithstanding the fact that more basic research has been done on the *akamushi* than on any other trombiculid mite, its geographical and its host distribution are still imperfectly known. From great areas in eastern Asia and the western Pacific, where natural conditions seem exceptionally favorable for it, no published records of its occurrence are available. Among such areas are most of the eastern part of China, the Philippines, Borneo, and most of the Celebes. As to the possible occurrence of the *akamushi* on several types of hosts, information is very scanty or wanting, particularly regarding amphibians, reptiles, and certain kinds of birds. It is believed that most of the published host and distribution records of the *akamushi* are included below. Names of authors reporting records are given in parentheses.

Published records of the occurrence of *Trombicula akamushi* (Brumpt): Formosa: On *Apodemus agrarius ningpoensis*, on house mouse, *Mus musculus*, on a musk shrew, *Suncus myosurus swinhoei*, on house rat, *Rattus norvegicus*, on *Rattus rattus rufescens* (Hatori); Kagi District (Zone of Mt. Ari) (Matsumoto). Japan: Akita Prefecture; Hondo, Echigo, on "field mice" (Hirst); Koshi District, River Shinanogawa, on "field mouse," on house rat, *Rattus rattus alexandrinus*, on house cat, *Felis domestica* (Kitashima and Miyajima); Niigata Prefecture, adults and nymphs taken (Miyajima and Okumura); Yamagata Prefecture, adults reared (Nagayo et al). Korea: Locality (?) (statement by Walch). Malay States (Selangor): Elmina, on *Rattus rattus jalorensis* (identified by H. E. Ewing); Sungei Buloh, on man, on *Rattus rattus diardii*, on *Rattus rattus jalorensis*, on *Trichys fasciolata fasciolata* (= ? *Atherurus macrourus*) (Gater); Pescadores Islands: Boko Island, on rodents and birds.

*The Distribution of Trombicula deliensis Walch*

According to our present knowledge, *Trombicula deliensis* Walch is confined to the tropical region of southeastern Asia, the East Indies, and northern Australia. Our knowledge of its host distribution is due largely to the work of Gater (1932).

Published records of the occurrence of *Trombicula deliensis* Walch: *Australia*: Queensland, coastal strip north of Townsville, on "rats" (Heaslip). *India*: Simla Hills (in northern India) (by Mehta). *Malay States* (all records by Gater): Selangor, Sungei Buloh, on *Rattus rattus diardii* and *Rattus rattus jalorensis*; Kuala Lumpur, on "rat"; Fraser's Hill, Pahang, Raub (Pahang), Port Dickson (Negri Sembilan). The material taken at these last four localities combined were from the following hosts: Man, *Paradoxurus hermaphroditus*, *Rattus concolor concolor*, *R. edwardsi ciliatus*, *R. mülleri validus*, *R. sabanus vociferans*, *Rhinosciurus laticau-*

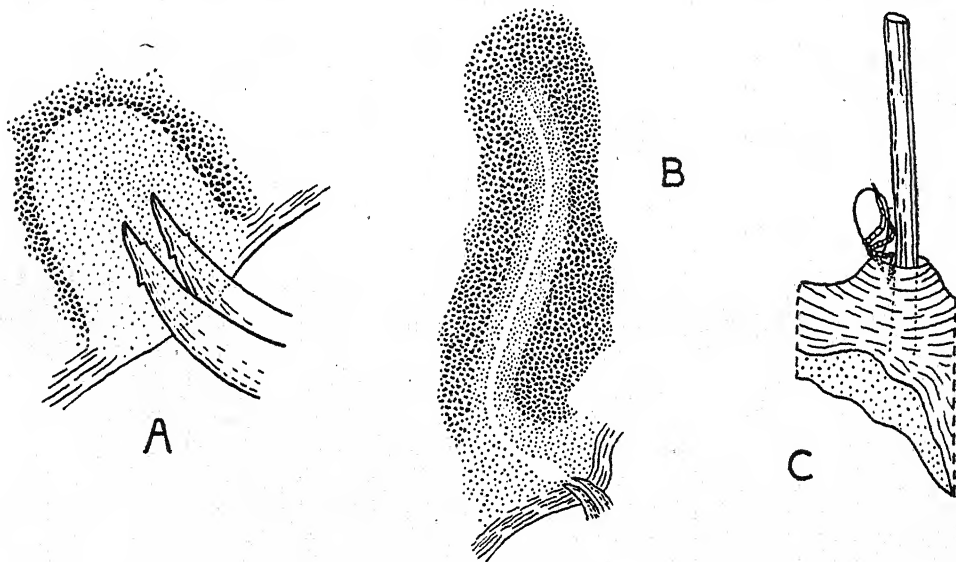


FIG. 7. At left, A, a longitudinal section of the "stylostome" in an early stage of development; B, a later stage in the formation of the same; C, "slice" of skin from calf of leg showing method of attachment of *Eutrombicula alfreddugèsi* in opening of a hair follicle. All much enlarged; A, original, B, after André, C, after Ewing.

*datus tupaoides*, *Sciurus prevostii humei*, *S. notatus miniatus*, *S. nigrovittatus bilitatus*, *Tragulus kanchil fulviventer*, *Trichys fasciculata fasciculata* (= *Atherurus macrourus*), *Tupaia glis ferruginea*. *New Guinea* (Territory of): Bulolo, on brown scrub rat, *Rattus leucopus mordax*, and Brown's rat, *Rattus axulans browni* (Gunther). *Sumatra* (all records by Walch): Goenoeng Rinteh (near Deli), on man; Lampong District, on man and "rat"; Limao Moengkoer (near Deli), on man.

## DIRECT INJURY TO HOSTS BY TROMBICULID LARVAE

Once on a host, trombiculid larvae do not attach immediately but run about until they find a fold in the skin, a place where the clothing is tight, or some other convenient place, such as the mouth of a hair follicle, for the insertion of their chelicerae. Of 26 attached individuals observed on the writer's skin (Ewing, 1921), 5 were attached inside the rim of hair follicles. The insertion of the chelicerae usually is un-

noticed by the human host; and it is only after the injection of a digestive fluid that itching is felt. This itching usually begins 3 to 6 hours after exposure.

The injection of this fluid produces a very characteristic effect. The tissue in immediate contact with it is liquefied, and the adjoining tissue becomes toughened. As the predigested liquefied tissue (not blood) is sucked up by the mite and more digestive fluid is injected into the cavity thus produced there is formed a sclerotized tube (Fig. 7, *A* and *B*) which may be as long as the total length of the mite itself. This tube has been called the "stylostome," and at one time it was thought to be a part of the mite. Very early the tube was described by Gudden (1871), who examined human skin with attached larvae of *Trombicula autumnalis*, and noted that the tubelike structure extended into the skin from the mouth parts of the mite. This he regarded as a structure of the mite itself, in which belief he was long followed by others. In more recent years Brèthes (1909), André (1927b), Feng and Hoeppli (1933), and others have shown that this tubelike structure or canal is formed by the reaction of the tissues of the host to the digestive fluid of the mite and is in no way an organ of the latter.

The pathological changes produced in the tissues adjacent to the canal have been studied in America particularly by Toomey (1921) and by Parkhurst (1937). The latter has summarized these changes as follows:

Between three and twenty-four hours after exposure the lesion becomes an urticarial papule and itches intensely. Generally this is the first manifestation noticed by the patient. It is often, but not invariably, follicular. In this lesion the larva, if still attached, appears near, or at, the center as a minute red dot. Within from one to three days the lesion becomes a pruritic, pale red, flat or conical papule—conical lesions often occur—and especially on the legs, thighs and lower portion of the abdomen these papules may become grossly hemorrhagic and deep purple. Some of the papules, whether hemorrhagic or not, may be surmounted by a pinhead-sized vesicle containing clear fluid. The itching is intense, generally being worse on the second or third day, and may persist after fourteen days. Owing to this the vesicle is generally scratched open and may be secondarily infected. If it is not excoriated it usually dries and is followed by scaling. Involution begins about the third day and is extremely slow. The papule shrinks, and pigmentation appears, persisting for from ten to fourteen days or longer. The lesions may be from 0.4 to 2 cm. in diameter and are sometimes larger. A lesion may last several weeks, during which time it may itch intermittently.

Secondary infection, which usually is due to scratching, may lead to a variety of conditions and may be very serious. According to Toomey (1921), in the Ozark Mountains and some other places where people go barefooted, some persons have lost toes as the result of secondary infection from chigger attacks over the toe joints.

#### TROMBICULIDS IN RELATION TO TSUTSUGAMUSHI DISEASE\*

Tsutsugamushi disease has probably existed in eastern Asia for many centuries as has been pointed out by Sambon (1928). He found mention of a fever agreeing in many respects with this disease as occurring in southeastern China in the Chinese medical work "*Pen ts'ao Kang Mu*" compiled in the sixteenth century. But it was in Japan where the disease first gained the attention of modern medicine. Here, along the flood plains of some of the rivers in certain northwestern districts, it has existed for a long time and has been studied for over half a century. Very early some of the clinical symptoms were well established, and from the beginning many people had claimed an association between the attacks of a small reddish mite larva,

\* The review here given is very brief, particularly considering the importance of the subject. For a more extensive review the recent paper by Farner and Katsampes (1944) may be consulted.

now known as *Trombicula akamushi*, and the disease. For this reason studies on the habits and the more specific relation of this mite with the disease were begun in the latter part of the last century. Along this line the work of Tanaka (1892, 1894, 1899) was outstanding. Work on the life cycle of the supposed mite transmitter soon followed and, according to Miyajima and Okumura (1917), Miyajima reared nymphs of *T. akamushi* as early as 1906 from larvae found attached to field rice. But it was several years later when Nagayo et al (1916, August) first reported the rearing of all stages of the mite. They give figures of these which show

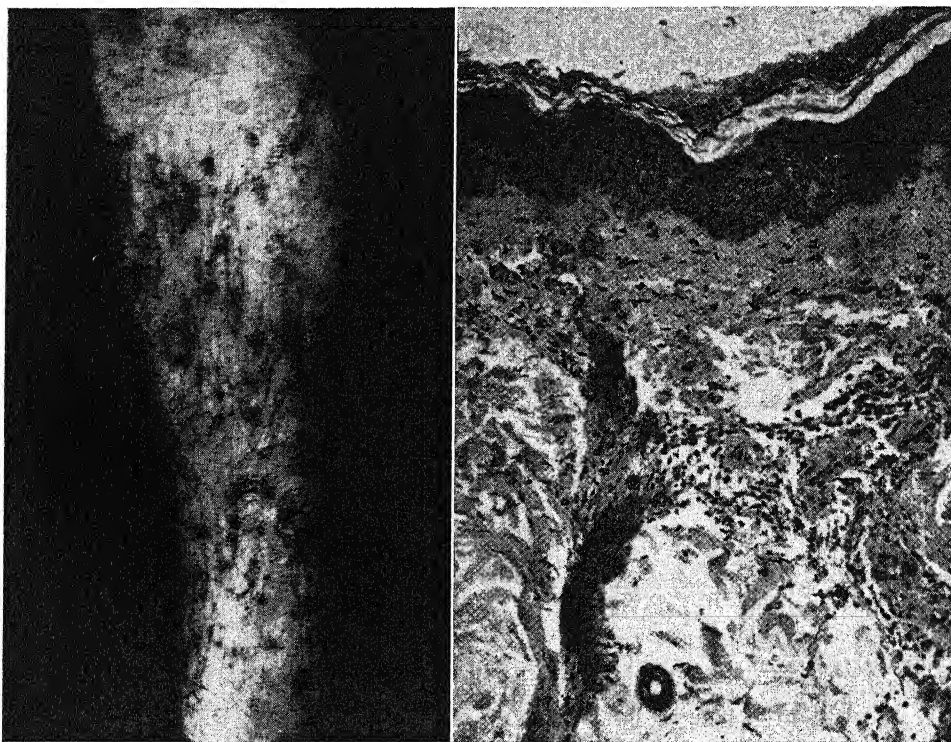


FIG. 8. Lesions produced by *Eutrombicula alfreddugèsi*. To left: Leg showing some lesions capped with the typical small vesicle; other lesions that have been excoriated by scratching (U. S. National Museum photograph). To right: Section from lesion on leg of man showing vascular engorgement in the middle portion of the cutis, lymphocytic perivascular infiltration and edema (after Parkhurst).

the true nymphochrysalis and also the egg. The egg, however, is photographed inside the body of the female and is very much larger in proportion to the size of the mite than the largest of the eggs shown by Kawamura and Ikeda (1936) (see Fig. 1, a). Independently two other groups of workers demonstrated the life cycle at about the same time, Kawamura and Yamaguchi (1916a) and Miyajima and Okumura (1917).

Hayashi (1920) was the first to describe the organism that is now regarded as the cause of tsutsugamushi disease, calling it *Theileria tsutsugamushi*. At the time he doubted its affinity with *Theileria* and suggested that further study would result in its removal from this genus. This was soon done by others.



Schüffner and Wachsmuth (1910) described a disease found at Deli, Sumatra, which was evidently very similar to tsutsugamushi disease. They called it Pseudotyphus, meaning in English pseudotyphoid fever. Later, Schüffner (1915) regarded this disease as only a variety of Japanese river fever (tsutsugamushi disease). Walch (1922) made a special study of the trombiculids attacking man in Sumatra and came to the conclusion that *Trombicula deliensis*, which he described as new, was probably the carrier of the disease which Schüffner has called pseudotyphoid fever. Walch and Keukenschrijver (1925) by means of injection of blood from a patient suffering with pseudotyphoid produced the disease in a gibbon, and by injecting an emulsion of 400 larvae of *T. deliensis* produced the disease in the same host.

Sellards (1923), apparently unacquainted with the work previously done by Hayashi (1920), obtained an organism in pure culture from animals infected with the virus of tsutsugamushi disease, which he named *Rickettsia nipponica*. Since his organism grew on an artificial medium it probably was not the same as the one described by Hayashi in 1920 as *Theileria tsutsugamushi*.

Kawamura and Imagawa (1931) found *Rickettsia* bodies in the salivary glands of a specimen of *Trombicula akamushi* taken from an infected field mouse. These they named *R. akamushi*, and claimed that the bodies were identical with those found in laboratory animals that had been injected with blood of a patient having tsutsugamushi disease. Hence they believed they had demonstrated the causative organism as well as the transmitter. Their *R. akamushi* should be regarded as a synonym of *R. tsutsugamushi* (Hayashi).

The occurrence of tsutsugamushi disease in Formosa was recognized early in the present century, and it has been studied there by several Japanese workers. Morishita (1942) states that the disease is widely distributed in the island, being found on the plains and in the mountainous regions alike. Although observed cases among the aborigines were few in number, he doubted that there was any difference in susceptibility due to race. The case mortality was found to average 12 per cent. The transmitter was said to be *Trombicula akamushi*.

In India, Boyd (1935), of the Royal Army Medical Corps, made an analysis of 110 cases of fevers of the typhus group reported for the year 1934. By means of serological tests 35 of these were placed in a "definite XK group." They corresponded closely to the Malayan scrub typhus, and apparently should be regarded as cases of tsutsugamushi disease. The vector was in no case identified. Fifteen of these 35 cases came from the Simla Hills, and a majority of them developed toward the end of the monsoon season.

Lewthwaite and Savor (1936) studied a typhus-like disease found in the Malay States. By means of cross-immunity tests they demonstrated the close similarity of scrub typhus with the Japanese tsutsugamushi disease. Also their experiments indicated the identity of the pseudotyphoid of Sumatra and the tsutsugamushi disease of Malaya.

The form of tsutsugamushi disease known as scrub typhus in New Guinea was investigated by Gunther (1935 to 1941b), who, however, usually referred to it as endemic typhus. He made a survey of the trombiculid larvae found in the areas where the typhus was endemic, noting in particular their host relationships. Several of the mite larvae were reared either to the nymphal or adult stage, and notes on their

biology were taken. By further noting the correlation or noncorrelation of the mite attacks with the development of typhus cases, he came to the conclusion that *Trombicula minor* Berlese (here believed to be in reality *T. hirsti* Sambon) was "the likely vector" and that a bandicoot, *Echymipera cockerelli*, was "probably the effective reservoir of the disease."

A disease of the typhus group similar to scrub typhus had been noted in northern Queensland, Australia. Heaslip (1941) regarded it as tsutsugamushi disease. His experiments indicated that the bandicoot, *Isodon torosus*, and one rat species of the genus *Melomys* and four of the genus *Mus* (*Rattus*) were probably reservoirs of the disease in nature.

#### PRINCIPLES FOR THE CONTROL OF TROMBICULID MITES

Studies on the biology of the trombiculid mites have been sufficiently advanced at present to make it possible to summarize advantageously certain principles for their control. Those that have given success, or at least some promise of success, are here stated.

##### *Destruction of the Mite Larvae in the Field*

Applications of sulfur or sulfur ointment directly to the skin in order to kill the trombiculid larvae are old and well-known practices. Owing to the fact that trombiculiasis and itch produced by *Sarcoptes scabiei* (Degeer) were long somewhat confused, the use of sulfur ointment against the latter doubtless on occasions destroyed the trombiculid larvae also. As early as 1873 C. V. Riley reported that "in some localities, where these pests most abound, sulphur is often sprinkled, during 'jigger' season, in foot-gear as a protection." The value of sulfur in destroying attached trombiculid larvae having thus been learned, their destruction in the field by the same agent was indicated. This method has been found effective (Chittenden, 1915). For the same purpose Smith and Gouck (1944) recently have shown that dusting with 2 per cent DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane) was effective. Comparing this insecticide with sulfur in effectiveness for the control of chiggers in the field, they state that DDT appeared best on the first day after application, sulfur on the seventh day, and the two equal on the third day.

##### *Modification of Ecological Factors in the Mite Environment*

Development of effective measures for controlling the trombiculids in their natural environment was long hindered because of our lack of knowledge concerning the free-living stages, and the native hosts. However, enough is now known concerning these to make possible the application of what may be termed "modification-of-environment" control. One of the ways in which the environment may be effectively modified is by destroying the growth of shrubs or larger herbaceous plants. Long ago, according to Washburn (1908), one Captain Zimmerman, who lived on an island in Lake Minnetonka, Minn., reduced the numbers of chiggers very materially by cutting out much of the underbrush and thus letting in the sunlight.

The removal of ground litter is a measure the value of which has been demonstrated. In the wooded areas near or about resort cottages along our Atlantic seaboard there are many situations where there is little brushy undergrowth, but there is a thick layer of ground litter giving good cover for trombiculids. Here the removal of this litter alone frequently is sufficient to bring about the drying of the sur-

face soil and thus the destruction of the nymphs and adults. This has been several times demonstrated along the west shore of Chesapeake Bay. As a supplementary measure it was found effective at a large Boy Scout camp (Ewing, 1927).

The use of infested fields for pasturage as a control measure is at times practical. Chittenden (1915) related that "correspondents and others" stated to him that chiggers had been destroyed in fields by turning in sheep.

Confining or removing important hosts during the active larval period of the mite's life cycle is a measure of restricted value. At one time during the development of our knowledge concerning the host relationships of *Eutrombicula alfreddugèsi* it appeared that the common box turtle, *Terrapene carolina*, was one of the very few important hosts. For this reason at the Boy Scout camp where attempts were being made to control the chiggers it was believed that if these turtles were caught and released far from camp, or placed in a pound during the active larval period, the numbers of chiggers would be greatly reduced. Accordingly (Ewing, 1927) this was done. The results were disappointing, and the practice was soon abandoned. Knowing what we now do concerning the many favored hosts of these trombiculid larvae, it is easy to understand the ineffectiveness of this plan. As a supplementary measure only, it may have had some value.

#### *Protection of Man Against Mite Attack*

Of all the measures used against trombiculid mites, those designed to protect man himself have been the most employed, and on the whole probably the most successfully. The measures come largely under three headings, the use of repellents on the skin, the impregnation of clothing with repellents, and the use of fine-mesh garments.

Some of the acaricides used to kill trombiculid larvae after they attach themselves to man have also been used as repellents. Especially is this true of sulfur and kerosene. Trombiculid larvae are very sensitive to many substances, particularly to oils, of which several kinds afford rather good protection.

Years ago, the writer (Ewing, 1925d) found that articles of clothing could be made mite-proof by impregnation with a suspension of flowers of sulfur in a strong, hot, naphtha soap solution. With this solution the following were impregnated: Wrap puttees, the legs of "coveralls," and underwear. But of particular value were impregnated "sleeves." The sleeves were made by cutting off the toe end of heavy stockings. Each of these "sleeves" was easily pulled on over the shoe into the desired position about the calf and ankle of the leg. In more recent years repellents such as dimethyl phthalate have been used effectively for treating the clothing. Concerning the use of this insecticide for treating clothing of members of our armed forces for the prevention of chigger attack, Madden, Lindquist, and Knipling (1944) state: "The most practical means of treating the clothing is to apply the liquid repellents from a small-mouth bottle in a half-inch band around the inside of the waist, the fly, and the bottoms of the legs of the trousers, the cuffs, fly, and neck of the shirt, and in a wide band around the upper part of the socks. Clothing treated in this manner with dimethyl phthalate, *Indalone*, or *Rutgers 612* gave good protection up to 30 days after treatment."

Closely woven garments will not permit the entry of trombiculid larvae through their mesh. Taking advantage of this fact, certain Japanese investigators devised

a mite-proof suit which prevents the entry of the larvae through any part of the outer clothing. Some of our so-called "coveralls" that cover the back and fit snugly about the neck can be used effectively to protect against the larvae by tying the bottom of each leg of the garment about the shoe top.

#### WARTIME INVESTIGATIONS OF TSUTSUGAMUSHI DISEASE IN THE SOUTH PACIFIC

When war with Japan broke out, already considerable ground work on the tsutsugamushi disease had been done in Australia and New Guinea, particularly by Heaslip (1941) and by Gunther (1935, etc). This work was now taken up by two United States agencies, one being a special Army commission, which was sent into the field by the Surgeon General of the U. S. Army, under the auspices of the United States of America Typhus Commission and the Army Epidemiological Board. The other was the Naval Medical Research Unit No. 2. The Army commission went to the South Pacific in September 1943 to study this form of tsutsugamushi disease in connection with our troop movements, and some of the early results obtained have already been published as we shall soon note. The Naval Medical Research Unit No. 2 was slowly organized in the latter part of 1943 and early in 1944 to study certain tropical diseases in the South Pacific, including tsutsugamushi disease. During the several weeks before the departure of members of its personnel the writer worked in coöperation with some of them at the United States National Museum.

During the present year a few articles have appeared dealing with tsutsugamushi disease in the South Pacific. Lipman, Byron, and Casey (1944) have reported on a clinical survey of 101 cases which they called scrub typhus that were admitted to the First Evacuation Hospital in New Guinea between February 8 and September 1, 1943. According to their report there was a case mortality rate of 7.9 per cent and an average duration of the febrile period of 17 days. It was found, however, that in 5 of the 8 fatal cases the patients also had benign tertian malaria.

Ahlm and Lipshutz (1944) soon followed with a report on about 70 cases of tsutsugamushi disease in an undisclosed (for reasons of military security) area, paying particular attention to the clinical symptoms but also noting other aspects of the disease. They found mites near sago palm swamps that were margined by kunai grass, though only occasionally could the mites be found where this grass grew tall in the natural clearings adjacent to the jungle. The authors were of the opinion that the field rat (species?) was the principal reservoir, but that other reservoirs, though of less importance, also were present. In regard to the primary lesion they state: "The most common location of the primary lesion of this disease is the scrotal area, though the inguinal and ankle areas are frequently involved." This lesion was not always produced. In fact many patients had exhibited no sign of it.

As to the seriousness of the disease Ahlm and Lipshutz state: "Malaria lends itself to treatment readily and has an exceedingly low mortality rate, with only about fourteen man days lost. Tsutsugamushi fever, on the other hand, lends itself to treatment very poorly and has a fairly high mortality rate (considering other figures), with at least one hundred man days lost."

For individual protection these authors recommend treating the lower part of the trousers with a repellent, wearing high boots or footgear, and dusting the body with a mixture of sublimed sulfur and talcum. They also state that the repellent now in use by "the U. S. Army is highly satisfactory if properly applied."



According to information prepared in the Office of the Surgeon General and U. S. A. Typhus Commission (Bul. U. S. Army Med. Dept. (76), pp. 52-61, May 1944), scrub typhus was encountered particularly in fields of kunai grass bordered by jungle along water courses, the danger of infection being greatest at the border of kunai grass and jungle.\* As control measures the preparation of camp sites by the cutting and removal or burning of the kunai grass was emphasized. Sleeping on the ground should be avoided, and tents should have floors raised 2 to 3 feet from the ground. Individual protection for personnel in combat areas should be obtained by bathing with a thorough application of soap as soon as possible after exposure, and by the use of repellents.

#### THE DANGER OF SPREADING TSUTSUGAMUSHI DISEASE BECAUSE OF WAR CONDITIONS

There has been considerable apprehension lest because of the present World War some of the tropical diseases, such as tsutsugamushi disease, may be introduced into many other countries where they are unknown today. Yet when we consider that tsutsugamushi disease has existed in eastern Asia for centuries without becoming established elsewhere, it might appear to some that even now there is little danger of its wide dissemination. However, it is observed that a similar rickettsiosis, Rocky Mountain spotted fever, apparently has spread from a small area in our Northwest to nearly every state in the union in less than half a century.

If tsutsugamushi disease is introduced into these other countries, in what manner will this probably take place? At first thought it might appear that soldiers returning from infested areas in the Pacific would be the most probable agent of introduction. Such an introduction might be brought about in two ways, first by the soldiers bringing with them attached, live, infected mite larvae, and second, by their bringing with them the Rickettsiae in their body tissues. In order to establish the disease in a distant country in the first case the attached larvae would have to engorge successfully and then drop into a proper ecological environment for the completion of their life cycle. Introduction by this method certainly is a possibility, but it should be noted that while man is a host readily attacked by these mites, yet he does not appear to be a favored host. At least that appears to be the case with our common chigger, *Eutrombicula alfreddugèsi*. I have allowed many of the larvae of this species to attach to my skin, and although some of them did engorge to repletion, from none could a nymph be reared.

Years ago Dr. Walch told me, while he was in Washington, that to his knowledge only a single nymph of any trombiculid had ever been reared from larvae that had engorged on man. He stated that from a larva that had engorged on a native in the Dutch East Indies a nymph was obtained. I did not learn the species, and it is possible that this very important observation was never put in print. If by chance these infected larvae should engorge successfully on man and also transform into nymphs, they yet would have to be in a proper ecological environment to reach maturity and transmit their Rickettsiae to the next generation.

In the second case a larva of a species not now a transmitter would have to attach to the soldier vector returned to his native country, and not only successfully engorge upon him but also acquire his Rickettsiae and drop off in a favorable ecological environment for the completion of its life cycle.

\* F. C. Bishopp, however, states that he recently observed islands adjacent to New Guinea devoid of kunai grass on which numerous cases of scrub typhus originated.

When all these various probabilities are considered, it appears that the chances are small that the disease could be widely spread by the agency of returning soldiers, either with attached, infected mite larvae or with the Rickettsiae in their bodies.

Another method of dissemination of tsutsugamushi disease might be by way of transportation of reservoir hosts other than man. Rats appear to be the most important of such hosts and their ability to spread from country to country and from continent to continent has been most convincingly demonstrated in the past.

Again a very real danger of spreading tsutsugamushi disease comes from the possibility of transporting infected mites, either in the nonparasitic stages or in the unattached parasitic stage. The nonparasitic stages are found in the surface soil. Hence, any shipment of soil from an infested area in eastern Asia or the South Pacific to countries outside these areas might contain one or more of these stages. Some of these could doubtless be transported alive and might establish themselves in a distant country under a suitable environment.

The free, unattached, parasitic larvae are resistant to heat and dryness and are very active. Their length of life unattached has not been accurately determined, but in the case of *Eutrombicula alfreddugèsi* it probably is several weeks. According to Lemaire (1867) the Mexican *tlalsahuatl*, now regarded as the same as our *Eutrombicula alfreddugèsi*, was introduced into France in 1867. The basis for his belief was the reported finding of a specimen in the eye of a girl, whose father had previously received from Mexico numerous boxes, the packing and contents of which were left for some time close to the lawn where the child played. In view of the facts that the position of the supposed attachment was unusual in this case and the specimen was lost before an identification of it could be made, this record cannot be regarded as authentic. However, a real danger exists, through shipments of various kinds, of introducing disease-infected trombiculids into distant countries.

#### PRESENT AND FUTURE PROBLEMS

Perhaps it would be fitting to end this discussion of the trombiculid mites and their relation to disease by pointing out some of the more important considerations relating to them that should require our immediate or future attention.

1. Doubtless of most immediate concern is the possibility of reducing the danger of disseminating tsutsugamushi disease because of the present war conditions. Although much more could be known concerning the biology and distribution of the mite vectors and the disease itself, enough is known to indicate that the prevention of widespread dissemination of this disease may be a difficult matter under World War conditions. Further study, however, may indicate some practical measure to be taken in the very near future to reduce the danger of spreading this serious malady.

2. In the line of investigation much attention should be given to the rôle of birds, particularly in regard to their dissemination of the mite agents and acting as natural reservoirs for the disease.

3. The need for a thorough morphological study of at least one economically important species of the TROMBICULIDAE is apparent. This should include the histology of the internal organs and should throw much light on the structure and function of the mechanism for secreting the extraintestinal digestive fluid. Also the reproductive system, which is known to be basically different from that of the harvest

mites proper, should be studied and described. Finally such a study might elucidate the manner in which the Rickettsiae are transmitted from the mother, through the egg, to the next generation of trombiculids.

4. The food habits of the nymphs and adults of all species should be investigated. Very little is known about those of any species.

5. The responses of the better-known trombiculid species to light, heat, moisture, and other elements of their ecological environment should be studied. This would give us a much sounder basis for developing certain types of control measures.

6. Finally, the whole problem of speciation in the trombiculids, from both a theoretical and a practical standpoint, should be undertaken by one or more investigators on a long-time basis, noting particularly the effects of hosts and geographical distribution.

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A NEW MOUSE FLEA, *PLEOCHAETOIDES BULLISI*, N. GEN. N. SP.,  
FROM TEXAS<sup>1</sup>

GUSTAF F. AUGUSTSON<sup>2</sup>

Incident to field studies on Bullis Fever,<sup>3</sup> some fleas were obtained from white-footed mice (*Peromyscus*) that are here illustrated and described as new to science.

Family DOLICHOPSYLLIDAE

*Pleochaetoides* new genus

Chaetotaxy of head like that of *Pleochaetis*: eyes moderately pigmented, small; labial palpus five (5) segmented; in males, tergal plate VIII large, upper and lower posterior angles of clasper small and about equal, sternite VIII reduced, with two large membranous flaps; chaetotaxy of tarsi, legs, and thoracic segments as in *Pleochaetis*.

Genotype: *Pleochaetoides bullisi* Augustson.

This new genus, as indicated, is closely allied to *Pleochaetis* Jordan (1933). Due to the present status and description of *Pleochaetis*, as given by Ewing and Fox (1943), a new genus was felt necessary to hold the fleas here reported on. Ewing and Fox (1.c.) reduced *Pleochaetis* to subgeneric rank under *Trichopsylla* Kolenati (1863). This latter was revived on the strength of Baker's (1904) type designation. In Kolenati's original description (1.c.) no mention was made of a pronotal ctenidium. Jordan (1.c.) recognized this when he erected *Pleochaetis*, stating, "the genus (*Trichopsylla*) was described as having no ctenidium on head and thorax." All species dealt with by Ewing and Fox (1.c.) under *Trichopsylla* possess at least a pronotal ctenidium. Baker's monographic work (1.c.), according to International Rules, may necessitate the continued use of *Trichopsylla*, as so designated, but the employment of established genera, as outlined by Ewing and Fox (1.c.), as subgenera within it appears illogical at present. If, and when, *Pleochaetis* is recognized as a genus by siphonapterists, the genus *Pleochaetoides* may perhaps be relegated to subgeneric status.

*Pleochaetoides bullisi* n. sp.

Holotype Male

*Head* (Fig. 1): Frontal tubercle small, acuminate; preantennal region with three (3) rows of bristles, eight (8) in upper row, three (3) in each lower row; eye small, not darkly pigmented; genal process sharply rounded; bristles on second segment of antenna reduced; postantennal region with three (3) rows of bristles (including submarginal row of occiput), three (3) in anterior row, five (5) in each posterior row; labial palpus slightly shorter than fore coxa; maxillae sharply acuminate.

*Thoracic and Abdominal Segments* (Figs. 2, 3 and 4): Pronotal ctenidium of sixteen (16) slender spines; tergal teeth present, small, two (2) on metathorax, two (2) on abdominal tergites

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<sup>3</sup> Livesay, H. R., and Pollard, M. Laboratory report on a clinical syndrome referred to as Bullis Fever. Am. Jour. Trop. Med. 23: 475-479. 1943.

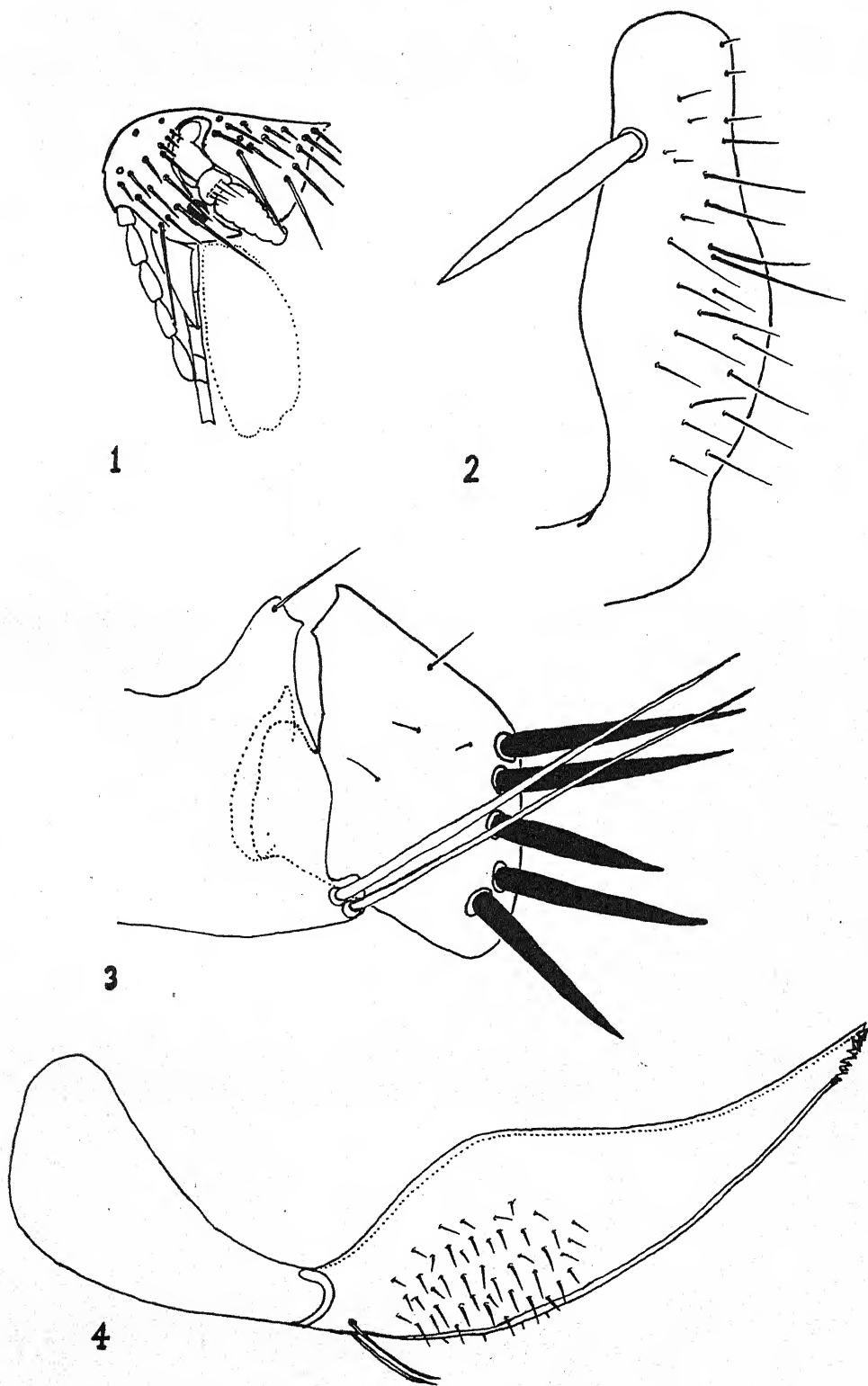
PLATE I

FIG. 1. *Pleochaetoides bullisi* Aug., head, holotype male.

FIG. 2. *Pleochaetoides bullisi* Aug., sternite IX, holotype male.

FIG. 3. *Pleochaetoides bullisi* Aug., finger, clasper, holotype male.

FIG. 4. *Pleochaetoides bullisi* Aug., sternite VIII, holotype male.



I to IV; a single, large antepygial bristle; tergite VIII very large, the postero-ventral margin of which joins sternite VIII; sternite X (XI of authors) reduced to small flaps; finger (Fig. 3) with rounded postero-ventral angle, tapered, narrow antero-dorsal angle, five (5) long, pointed spiniforms, the middle member slightly shorter than others; clasper (Fig. 3) with two (2) long acetabular bristles, which are situated postero-ventral to acetabulum on a narrow extension of the ventral margin of the clasper, process of clasper narrow; sternite IX (Fig. 2) broad, not divided into lobes, a single prominent, modified bristle in the upper anterior half, with numerous small setae in the lower posterior half; sternite VIII (Fig. 4) apparently reduced to a small basal sclerite, with two (2) large membranous flaps; penis long and slender, spring with one full turn.

*Legs:* Tarsus V with five (5) lateral plantar bristles, the first pair distinctly displaced medially; length of hind tarsus I not exceeding II and III together; coxa II with long, thin bristles in apical half only; outer surface of fore femur with seven (7) small setae.

*Holotype:* A male, collected by the writer and Dr. J. M. Brennan at Camp Bullis, Bexar County, Texas, 1 June 1944, from *Peromyscus pectoralis laceianus*. Deposited in the U. S. National Museum, Washington, D. C.

*Paratype:* A male, collected as above, retained in the Eighth Service Command Laboratory, Fort Sam Houston, Texas.

*Type Host:* *Peromyscus pectoralis laceianus*.

*Type locality:* Camp Bullis, Bexar County, Texas.

*Remarks:* This new flea is named after the locality from which it was recovered. Host determination is that of Dr. L. Dice, Department Vertebrate Zoology, University of Michigan.

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## PHYSIOLOGICAL RESPONSES OF *NEOECHINORHYNCHUS EMYDIS* (ACANTHOCEPHALA) TO VARIOUS SOLUTIONS

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Various writers have called attention to the fact that the body of a normal, living acanthocephalan is distinctly flattened and somewhat wrinkled (Hamann, 1891; Looss, 1901; Van Cleave, 1941; et al). Attainment of a turgid, cylindrical form in these worms is attributable to the operation of two unrelated sets of factors, one of which results in a normal phenomenon of plumping and the other is explainable as a physiological response to unfavorable change in the environment. The development of eggs in the fertilized female produces a normal, functional alteration of body shape, causing the body of the gravid female to assume a distended, turgidly cylindrical form. Practically all preserved adult and post-larval individuals of both sexes and in all stages of growth have this same cylindrical form, attributable not to normal, functional adjustment but to physiological changes which take place as a response to abnormal or unnatural media.

The importance of the body covering in the life processes of the individual is particularly significant in this group of parasitic worms. In the complete absence of all organs for digestion, the acanthocephalan depends entirely upon the permeability of its body wall for admission of all metabolic materials and, with exception of a few species which have specialized excretory organs, the body wastes must likewise pass outward through the body covering.

One of the most distinctive features of the subcuticula, which comprises by far the greatest bulk of the body wall, is a series of intercommunicating spaces and canals. At least some of these are united into continuous vessels.

While extensive experimentation has been conducted on maintenance of cestodes, trematodes, and nematodes *in vitro*, only one paper dealing specifically with ACANTHOCEPHALA has come to the writers' attention. This is the brief study by Gettier (1942). In that paper no mention of the response of the worms to the various media is given, except as length of survival in each medium is recorded.

The maintenance of a collapsed body by ACANTHOCEPHALA within their normal habitat, followed by distortion on removal to water or histological fixatives, suggests the desirability of determining the effects of various media on state of the body. This problem immediately finds direct application in the study of media used for maintaining ACANTHOCEPHALA for experimental purposes outside the body of the host. In testing media, the length of time that a worm will survive should at the same time be supplemented by observations on the normal or abnormal conditions induced by the medium.

Beginning in 1942, the junior author of this paper performed a series of tests to determine the effects of various media on *Neoechinorhynchus emydis* (Leidy, 1851). This species was selected because of its availability throughout the year and because its relatively small size ensures convenience in handling. Since the adult worms live for many months in the turtle intestine, even when the host is not feeding, a continuous supply was assured.

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In the present study attention was directed primarily to maintenance of normal body conditions and to the environmental factors which cause alteration of body form. Earliest observations on body form had given ample evidence that many media produce turgidity of worms *in vitro*. Several series of experiments were devised to determine the effects of various solutions on body form. As a further program, a series of experiments was arranged to determine the possibility of restoring normal body form after worms had become turgid. As a check to these studies *in vitro*, an extended series of experiments was performed to determine the possibility of altering, at least temporarily, the environment in the host intestine so as to induce changes *in vivo* paralleling those produced *in vitro*.

The most pronounced effects upon *N. emydis* were observed when worms from a freshly opened turtle intestine were placed in tap water. From a fairly long series of trials, no individual remained alive for more than 6 hours in tap water and in every instance the body became turgidly cylindrical in less than an hour after being placed in the water. In order to check the otherwise uncontrolled factors regarding the condition of the worms (due to relative age, their physiological states, or the condition of the host) several experiments were performed in which some of the worms from the same host individual were placed in tap water at the same time that others were introduced into various other media, as discussed later in this paper. Invariably, those individuals in the water died in 6 hours or less while those in salt solutions lived a longer period. The length of the survival and shape of the body were correlated with the strength of the solution.

Gettier (1942) found that 0.5% sodium chloride to which small amounts of calcium chloride had been added kept specimens alive on the average of 20.3 days. In the present series of experiments, the solution most used was 0.5% sodium chloride to which 0.02% calcium chloride had been added. Of several hundred worms subjected to this treatment, most individuals retained their flattened, normal condition for from two to four days, after which they became turgid and lived for total periods of from 5 to 20 days outside the body of the host. While the worms continued to live for relatively long periods in this hypotonic solution, they soon lost normal appearance (in from 2 to 4 days) and consequently they were in distinctly abnormal condition for most of the period of their survival.

The rather striking individual differences in length of time that the worms survived in experiments under identical treatment emphasizes the fact that the physiological state of the worms at the outset of the experiment could not be determined.

Change to fresh medium and handling of vials and Petri dishes incident to the frequent observations on the worms seemed to be responsible for reduction in the survival time for many individuals. A series of preliminary experiments gave evidence that these parasites live longest in media from which contact with free atmospheric air is excluded. This was accomplished by completely filling the container with medium and then loosely stoppering with a cork to exclude all air space.

Two experiments with *N. emydis* in frog Ringer's produced worms which were very active for the first day and remained fairly active for from 3 to 6 days. After about the sixth day their bodies became greatly distorted and turgid and they remained in this misshapen, inactive condition for from 4 to 8 days when they died, after a total of from 10 to 14 days in frog Ringer's. Judging from the swelling of the worms, frog Ringer's is hypotonic to *N. emydis*. Although the balanced salt

solution maintains life for fairly long periods, the worms soon become abnormally swollen and distorted.

It is clear from the foregoing that solutions previously recommended for physiological work with turtles and for maintaining *ACANTHOCEPHALA* outside the body of the turtle host have extremely adverse effects on *Neoechinorhynchus emydis*, causing the body to become inordinately distended and turgidly cylindrical. Since normal saline solutions are so commonly recommended as a medium in which to examine living worm parasites and as a bath for removal of mucus and other foreign material preliminary to fixation, the writers decided to test the effects of higher concentrations of sodium chloride on *N. emydis*.

Specimens transferred directly to 0.85% sodium chloride for washing and then placed in a fresh stock of this same solution, retained their flattened body form, with considerable wrinkling of the surface, for 12 of the 14 days of their survival outside the body of the host. In yet another experiment with 0.85% sodium chloride, the worms remained flat 13 of the 15 days of life in the medium. In 0.8% sodium chloride, specimens became turgid in approximately 10 days and died on the tenth day. These results indicate that *in vitro*, solutions of from 0.8 to 0.85% sodium chloride are essential for maintaining the normally flattened body form of *Neoechinorhynchus emydis*.

In several experiments with 0.75% sodium chloride, the worms survived for from 9 to 13 days although they became distinctly turgid on the second to the fourth day and remained in that condition for the remainder of their lives. When specimens were placed in 0.7% sodium chloride, few of them lived more than 10 days and all became turgid on the third or fourth day, showing that the body wall in this lower concentration could not function normally as a membrane to control the passage of liquids through it.

The readiness with which the body of *N. emydis* responds to hypotonic solutions, changing from its normal, flattened form to turgid, cylindrical form, offers opportunity to examine the possibility of reversing this plumping reaction. With worms once plumped, *in vitro*, is it possible to restore them to their normal form? In one experiment, worms removed from the intestine of a turtle were placed in tap water, where they were allowed to remain for one hour. At the end of this time they were highly turgid. Upon transfer from water to 0.85% sodium chloride, they soon began to lose their turgidity and within five hours had become fully flattened. Apparently they were not seriously affected by the sudden changes in the medium for in this and other experiments some individuals survived for 8 to 9 days. However, in one experiment the alteration back to normal body form was not permanent. Three days after the removal from water to 0.85% sodium chloride the bodies became turgid again, even in the hypertonic solution. Although some of these last-mentioned individuals survived a total of 9 days, normal body permeability was obviously somewhat modified.

The readiness with which living specimens of *N. emydis* alter their body form when subjected to hypotonic solutions *in vitro*, offered a challenge to investigate the possibility of changing the body form while the worms still remained normally attached within the intestine of the living turtle. Solutions identical with those used in the series of *in vitro* experiments were introduced into the intestine of the turtle by means of a tube inserted down the oesophagus of the living host. At varying

intervals following the oral administering of fluids, the turtles were autopsied in order to observe the effects of the media introduced upon the body form of the worms *in situ*.

In an experiment (Dec. 20, 1943), 20 cc of dechlorinated tap water were introduced into the digestive tract of a turtle. Upon autopsy one hour later all of the worms, even the smallest and most immature males, were in a turgid state. Some of the worms removed to tap water in a Petri dish survived for only about 3 hours, others placed in 0.5% sodium chloride and 0.02% calcium chloride lived for periods ranging from a few hours to 2 days.

Oral injection of 20 cc of 0.8% sodium chloride was followed by autopsy after 1.5 hours. None of the worms were turgid and, furthermore, there was no evident bodily distortion in the bodies of worms removed to the same solution for the 10 days of their survival. Similar results followed oral injection of 0.85% sodium chloride. When removed from the intestine to 0.85% sodium chloride in a Petri dish, the bodies remained undistorted for the full 12 days of life outside the host. In one experiment, a single hour elapsed between administering the 0.85% solution and the autopsy, in another,  $4\frac{1}{2}$  hours, but there was no observable difference in the response of the worms in the two instances.

To test the possibility of altering and subsequently restoring the body form of worms still within the unopened intestine of the host, parallel to the series of experiments *in vitro*, a turtle was given an oral injection of tap water. Upon autopsy, after two hours, 15 worms were observed, all attached to the intestine wall but all turgidly cylindrical in form. As these worms were removed from the host they were separated into four different groups each of which received different treatment. The turgid worms which were removed to 0.70 and 0.75% sodium chloride remained turgid while those placed in 0.80 or 0.85% sodium chloride regained their normal body form. It is thus clear that the response of worms subjected to the influence of water in the turtle intestine followed by removal to salt solution, is in all essential respects parallel to the response observed when the experiments were conducted wholly *in vitro*.

The normally flattened bodies of males and non-gravid females of *Neoechinorhynchus emydis* are readily altered by changes in the surrounding medium. Hypotonic solutions cause the body to become inordinately swollen and turgidly cylindrical. Even changes in the content of the host intestine may induce changes in body form of the living worms attached within the intestine. Radical changes in body form induced by water or hypotonic solutions, either *in vitro* or *in vivo*, cause the death of the parasites.

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## STUDIES OF SHEEP PARASITES. IV. SURVIVAL OF SHEEP NEMATODES ON PASTURE DURING THE FALL MONTHS\*

PHILIP A. HAWKINS, C. L. COLE AND E. E. KLINE

The length of life of the infective larvae of the strongylids is of both practical and theoretical importance. To the animal husbandryman it determines how long pastures must be vacated until live stock may be safely returned, to the helminthologist it explains the activity of these larvae under natural conditions. Studies of this type have been sadly neglected until recent years.

Ransom (1906) found that the larvae of *Haemonchus contortus* showed little reduction in the number of food granules when kept out of doors from December 27 to March 22. Ransom (1908) also reported that infested pastures will not become free of stomach worm infection between October 25 and June 16 at Washington, D. C. However, this writer later (1910) reported that if a pasture was free of sheep for a year it would practically free itself of stomach worm larvae. Although Veglia (1915) showed that the infective stomach worm larvae were short lived the conclusions of Ransom have been generally accepted. These conclusions as to the longevity of stomach worms *H. contortus* have in general been confirmed by Dikmans and Andrews (1933), Kammlade, Graham and Boley (1936) in Illinois, Boughton and Hardy (1937) in Texas and Baker (1939) in New York. It is probable that the test lambs of Ransom (1906) and Kammlade, Graham and Boley (1936) were infected before being placed on pasture. However, Veglia (1915) and Mönning (1930) showed that most of the trichostrongylid larvae were not capable of surviving more than 2-3 months in the dry weather of South Africa. Griffiths (1937) and Swales (1939 and 1940) showed in Southern Canada that pastures infested with *Haemonchus contortus* and *Oesophagostomum columbianum* were freed of infestation over the winter months, although other trichostrongylid larvae were able to survive in small numbers. Shorb (1943) at Beltsville, Maryland, found there was no survival on grass plots of *Haemonchus contortus* from December to April. Shorb (1944) noted in a study of infested grass plots that a few infective larvae were capable of surviving from August or October to the following April or May but these were sluggish, vacuolated and probably non-infective. Dinaburg (1944) also reports on the short life of *H. contortus* larvae and their failure to live over the winter. Sarles (1943) also showed there was no carryover on pasture in Maryland. Sarles (1943) demonstrated the overwinter loss of nodular worm larvae from a sheep pasture at Beltsville, Maryland. Rebrassier (1933) in Ohio reports in an abstract that nodular worm larvae are capable of surviving at least one winter on pasture. Shorb (1942) reported that on summer pastures in Maryland all the strongylid parasites of sheep were destroyed in 2½ months, and that even an abundance of shade probably does not protect materially these preparasitic stages. Shorb also showed that there was no development to infectivity during the late fall and winter, but that small numbers of *Ostertagia*, *Trichostrongylus* and probably *Nematodirus* survive the winter and are infective in the spring. Griffiths (1937)

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also showed that *Ostertagia*, *Trichostrongylus* and *Nematodirus* would survive the winter in southern Canada. Kates (1943) reported that there was no evidence of survival of *Oesophagostomum* or *Bunostomum* on pasture over the winter, but that *Trichostrongylus* and *Haemonchus* were capable of survival in small numbers and *Nematodirus* and *Ostertagia* in moderate numbers. Therefore, it is apparent at the present time that there has been a reversal of Ransom's earlier conclusions on the survival of *H. contortus*. It is probable that *Haemonchus contortus*, *Oesophagostomum columbianum* and *Bunostomum trigonocephalum* are not capable of surviving the winter months under most conditions and that *Ostertagia*, *Trichostrongylus* and *Nematodirus* may survive in small numbers. During the dry summer months it is probable that all the infective larvae are killed in 2 or 3 months.

#### METHODS

The lambs used in this experiment were removed from the ewes at birth and bottle-fed cows' milk. They were kept in screened box stalls, bedded with straw, and the pens cleaned twice each week. The lambs had free access to alfalfa hay, oats, water and salt. Repeated fecal examinations did not reveal the presence of any parasitic infection except coccidia and *Strongyloides*.

The experimental pasture was alfalfa-brome grass and about  $\frac{1}{4}$  acre in size. There was no shade on this plot. A group of untreated ewes and their lambs had access to this land from May 22 to September 1. This group was infected with *H. contortus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Oesophagostomum*, *Chabertia*, *Trichuris ovis* and *Moniezia expansa*. Parasite-free lambs were placed on this pasture for two weeks, removed to a screened straw bedded box stall for three weeks, killed, the nematodes removed, counted and identified.

#### RESULTS AND DISCUSSION

Weather data for the period tested are presented in Table 1. The number of nodules found on the gastro-intestinal tract and the number and species of worms

TABLE 1

	Temperature			Precipitation		Solar and sky radiation, gram calories/cm <sup>2</sup>
	Mean	Maximum	Minimum	Total inches	Snowfall, inches	
September .....	57.5	86	33	3.45	0.0	8,833.7
October .....	48.4	77	32	1.80	1.7	6,173.5
November .....	35.1	64	19	2.16	3.1	3,137.1
December .....	25.4	48	-3	0.45	1.9	3,455.9
January .....	28.2	63	3	1.32	2.3	3,225.7

Courtesy of the U. S. Soil Conservation Service Weather Station, 100 yards from pasture.

TABLE 2

	September 12	October 3	November 4	December 23	January 15
Nodules .....	15	51	4	0	0
<i>H. contortus</i> .....	43	26	0	0	0
<i>Ostertagia</i> .....	392	181	4	42	102
<i>Cooperia</i> .....	592	320	279	80	} 1000
<i>Trichostrongylus</i> .....	112	35	46	22	
<i>Nematodirus</i> .....	696	1143	1325	682	
<i>O. columbianum</i> .....	0	1	1	0	0
<i>Chabertia ovina</i> .....	1	8	1	0	0
<i>Trichuris ovis</i> .....	2	74	3	63	8
<i>Moniezia expansa</i> .....	7	0	2	1	0

recovered are presented in Table 2. Unfortunately the specimens found in the small intestine for January 15 were accidentally discarded so that only the total number of worms is presented. However, it is known from fecal examinations that *Trichostrongylus* and *Nematodirus* were present, whether *Cooperia* had disappeared cannot be stated although the eggs were absent.

*H. contortus* larvae were killed on the pasture in two months or less as shown by the fact that by November 4 infection of lambs failed to take place, and no infection with this species was noted during December or January. *O. columbianum* was greatly reduced in number after 2 months and had disappeared in 3½ months. *Chabertia ovina* larvae also had been destroyed on the pasture in 3½ months. However, in 4½ months the infective larvae of *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Nematodirus* and *Trichuris ovis* were still alive and capable of infection. Whether the larvae of *Cooperia curticei* were still alive after 4½ months unfortunately could not be determined, although they were still present at 3½ months.

In this area the common stomach worm and nodular worm are of greatest economic importance, therefore it was gratifying to note that they disappeared so rapidly from the pasture. Permanent pastures in the generally accepted sense of the term cannot be blamed for perpetuating these parasites from one year to the next. Hawkins et al (1944) have shown that a small residual infection is carried over the winter months in the ewe. This infection is multiplied by the ewe in the first warm weather of spring, and the lambs finally become infected in the early summer. It is interesting to note that there is a marked age resistance in the lambs preventing their early acquisition of infection.

Therefore, it appears that under the climatic conditions of Michigan the most important parasites economically, *H. contortus* and *O. columbianum*, are not perpetuated by the pastures, but by the breeding flock.

#### SUMMARY

1. Pastures infested with *Haemonchus contortus* are apparently freed of infestations in 2 months in the late summer and early fall; with *Oesophagostomum columbianum* and *Chabertia ovina* in 3½ months.
2. *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Nematodirus* and *Trichuris ovis* larvae were still viable after 4½ months on pasture.
3. The breeding flock appears to be of greatest importance in perpetuating parasites.

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## DEVELOPMENT OF A POWDER TREATMENT FOR THE CONTROL OF LICE ATTACKING MAN

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Military personnel are especially subject to infestation by one or more species of lice. The body louse, or "cootie," *Pediculus humanus corporis* Deg., has been incriminated as a vector of several important diseases, such as typhus fever, trench fever, and epidemic relapsing fever. The head louse, *P. humanus humanus* L., is also considered capable of transmitting these diseases. The crab louse, *Phthirus pubis* (L.), is not known to carry any disease, but the irritation produced by an infestation can become serious in some individuals.

Because of the tremendous importance of typhus in military operations,<sup>3</sup> research was undertaken to develop a powder for the control of body lice. Such a powder should be nontoxic to man when in contact with the body for long periods. Not only should the powder kill lice present at the time of treatment, but the treated clothing should remain effective in killing lice for several days. This residual effect was particularly necessary so that in the delousing of a unit, which might require several days, treated men would not become reinfested by contact with infested individuals. If the treatment did not remain effective beyond the incubation period of louse eggs, it was desirable that an ovicide be incorporated to destroy as many eggs as possible.

This report outlines the general procedures used in the louse investigations and summarizes the tests which led to the recommendation of the MYL powder for use by the armed forces. The work was begun in the spring of 1942 at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine. Another powder has since been developed (Bushland et al 1944), which will be discussed in a subsequent article.

### TEST METHODS

The body lice used in the insecticidal tests were taken from an incubator colony<sup>4</sup> reared in a manner modified from that described by Moore and

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<sup>1</sup> Now Captain, Sanitary Corps, Army of the United States.

<sup>2</sup> The work described in this paper was done under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine. The authors are indebted to W. E. Dove, chief of the Division of Insects Affecting Man and Animals, who was located in Orlando during the early stages of the work.

<sup>3</sup> Appreciation is expressed to Colonel W. S. Stone, of the Office of the Surgeon General of the Army, who strongly urged that research be undertaken to develop an insecticide, preferably a powder, that could be used readily by the individual soldier to control body lice, and whose active interest in this project greatly encouraged the authors.

<sup>4</sup> The parent stock consisted of a mixed collection made in Washington, D. C., by W. E. Dove and W. G. Bruce, and in Orlando by the senior author. During the period of this work the colony was maintained by G. H. Culpepper and N. B. Carson.



Hirschfelder (1919). The colony was held at a temperature of  $30^{\circ} \pm 2^{\circ}$  C in fairly humid incubators (approximately 85 per cent), and the lice were removed twice daily for feeding on human hosts. The maintenance of this colony has recently been described (Culpepper 1944). Recently fed young adult lice were used in all laboratory tests.

*Beaker tests.*—Since hundreds of organic compounds were to be evaluated as toxicants for body lice, it was necessary to devise a rapid means of eliminating ineffective materials. In the first elimination procedure test materials

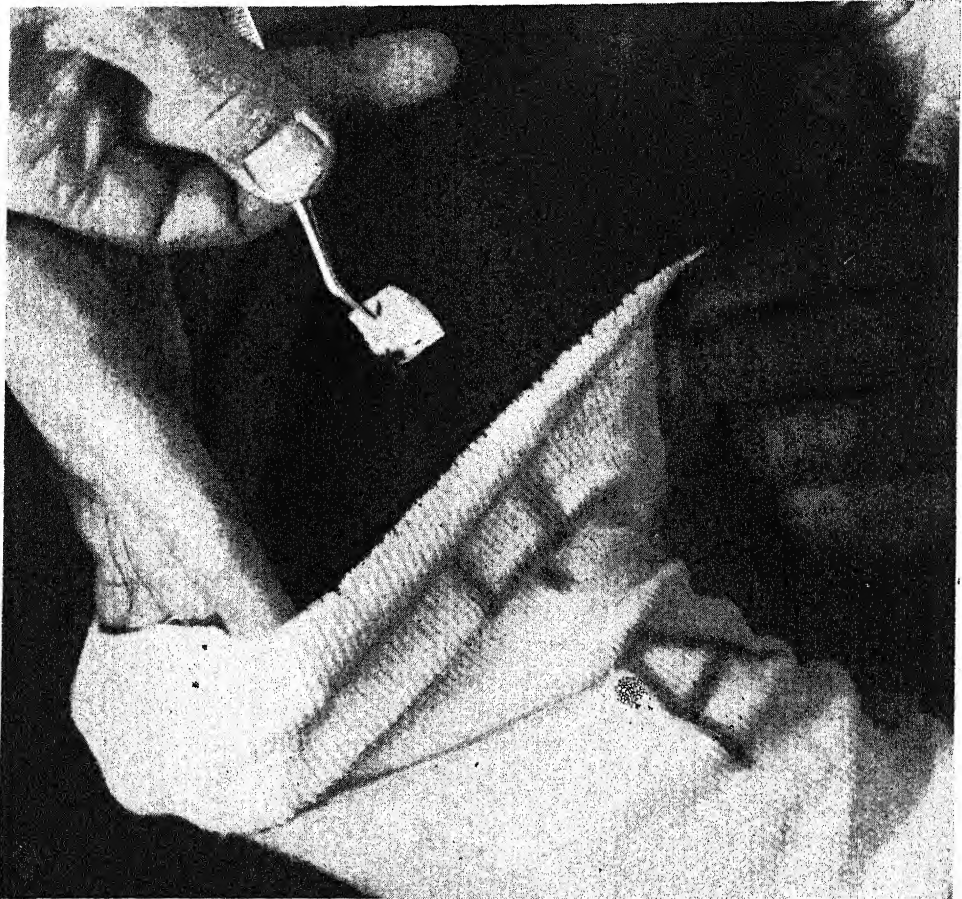


FIG. 1. Introduction of lice into sleeve in arm-and-leg test.

were dissolved at a concentration of 1.0 per cent in a suitable solvent, usually acetone but in some cases benzene, chloroform, or other solvent. Pieces of woolen cloth about 1 inch square were immersed in the test solution and then dried at room temperature for 2 hours to remove the solvent. The impregnated pads were then placed in 50-ml beakers and 10 lice introduced. The test beakers were held under the same conditions as was the louse colony. Since the instinct of lice is to cling to cloth, if the chemical with which the cloth was impregnated was sufficiently toxic, the lice were killed. Mortality records were taken after 24 hours. If a material was toxic, lice were added at

intervals to determine the duration of effectiveness of the treatment. Untreated controls were present in all tests, and it was found that, in the absence of an insecticide, lice survived the test conditions for 24 hours with negligible mortality.

*Arm-and-leg tests.*—Compounds found to be toxic in the beaker test were next tested as powders. The substances were deposited on pyrophyllite from an acetone solution to obtain powders containing 5.0 per cent of test material. These powders were evaluated by a method designated as the "arm-and-leg test."

In this test powders were spread uniformly over the inner surface of balbriggan cloth sleeves and legs at a dosage of 3.0 grams per garment, and rubbed into the fabric with the fingers. The garments had an area of about 65 square inches, and the dosage used was equivalent to about 2 ounces of powder per suit of winter under-



FIG. 2. Arm-and-leg treatments on subjects.

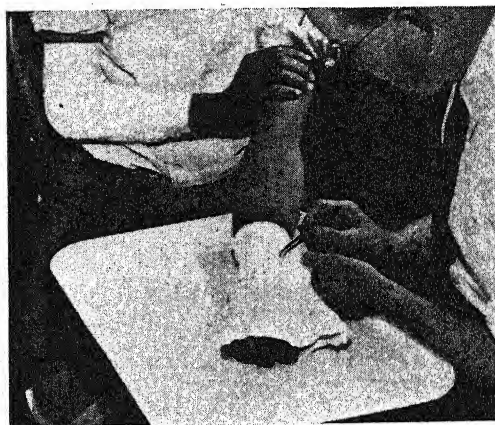


FIG. 3. Examination of lice after exposure in treated sleeve.

wear. A given material was applied to at least two, and usually four, treated sleeves or legs. The treated garments were placed on the arms and legs of research subjects and the lower openings fastened to the skin with adhesive tape. Twenty-five body lice on a small cloth pad were placed in each garment, and the top was sealed with tape. Figs. 1, 2, and 3 illustrate the method.

During the interval between introduction of lice and subsequent examination, the subjects were allowed to continue their normal activities. Examinations were made after the garments had been worn 24 hours. If the treatment was effective the garment was reinfested. Reinfestation and examination were repeated at daily intervals for 3 days and thereafter every 2 days until the treatment failed to kill all the lice. In some cases, for comparative purposes, tests were continued until a large percentage of the lice recovered were found to be normal.

At intervals untreated garments were used as controls, and frequently all the 25 lice were found at the time of examination. On an average, however, about 20 normal lice were recovered after 24 hours' confinement under untreated garments. Some of the insects were accidentally crushed in the host's

normal activities. Rarely did any escape, and the research subjects underwent the tests without fear of becoming infested.

The results obtained by the arm-and-leg method are not considered to represent those that would be attained under natural conditions. In these tests the garments were more heavily treated than in recommended practice and, since the lice were confined, they could not migrate to untreated areas. The treatments gave a greater degree of effectiveness than could have been obtained in practical louse control, but it is believed that materials that failed in these tests could not have succeeded under natural conditions.

By this method treatments could be quite accurately compared, since four materials could be tested simultaneously on one man. By replicating the treatments on several men and rotating the position of the treatments, rather accurate evaluations were made.

In addition to being used for tests of powders, the sleeves and legs were also used in impregnation studies wherein they were first dipped in or sprayed with solutions of insecticides. When dry, the impregnated garments were tested in the usual manner.

*Tests of ovicides.*—Louse eggs for ovicide tests were obtained from the stock culture. In the routine rearing procedure ovipositing lice were kept on woolen cloths about 1 inch square, and these pads were changed every 2 days. Thus a freshly removed pad contained eggs varying in age from newly laid to 2 days old, and pads with eggs near the point of hatching also contained eggs within 2 days of hatching. In the original tests these two age groups were used, but more recently only eggs 4 to 6 days old have been used for ovicide tests.

The materials were evaluated as powders and as solutions. To test a powder a pad bearing approximately 200 eggs was placed in a small paper bag (one-fourth pound size) with one-half teaspoonful (approximately 1.2 grams) of a pyrophyllite dust containing the desired concentration of ovicide. The sack was shaken vigorously for about a minute and the pad removed. Treated pads were held in a cabinet at a temperature of  $30^{\circ} \pm 2^{\circ}$  C and a fairly high humidity (usually 85 per cent) for incubation. After hatching was completed in the controls (similarly dusted with plain pyrophyllite), records were taken, examinations being made under the low power of a dissecting microscope. Solutions were tested by immersing the egg pads for 5 to 10 seconds in an alcohol or acetone solution of the test material. Neither of these solvents is ovicidal. The treated pads were held in a well-ventilated room until the solvent evaporated and were then handled according to the technique described above for powder treatments.

An estimate of the ovicidal effect of a treatment being tested by the arm-and-leg method was also obtainable merely by placing a pad bearing louse eggs within the treated garment.

The laboratory technique described served to eliminate chemicals having little or no ovicidal value. Promising materials were tested further on research subjects infested with eggs.

*Tests on grossly infested men.*—Experiments with materials that had shown promise in laboratory tests were conducted in a dormitory where men were domiciled during the period required to obtain the necessary data. The subjects were artificially infested with large numbers of lice and eggs to simulate conditions of heavy

natural infestation. These tests served to eliminate many of the materials found promising in arm-and-leg tests.

Each man was artificially infested by introducing adult body lice, usually 500, on his clothing, about 6 hours before the treatment was applied. The time between release of the lice and application of the treatment allowed the lice to migrate to all parts of the garments and to become at least partially established. Further to simulate natural infestation, woolen patches containing several hundred eggs in different stages of incubation were clipped to the garments. Just before the treatment was applied, the numbers of lice present on the underwear, outer clothing, and in the bed of each subject were recorded.

Except in special cases, all the tests were conducted with knitted cotton, winter-weight union suits with long sleeves and legs. The outer garments were of the usual type worn in this climate. In the tests of powders the dosage was 30 grams per suit of underwear. Powders were applied by carefully sprinkling from a shaker-top can over the entire garment and then lightly rubbing into the fabric. Only the inside of the underwear was treated. The outer clothing and the socks worn by the subjects, and their beds, were not treated. In actual practice under field conditions, however, it would be necessary to treat at least the seams of the outer clothing, the socks, and the beds of persons exposed to severe infestations of the body louse.

The degree of control obtained with each material tested was based on daily observations following the treatment. The underwear, the outer clothing, and the bed of each subject were thoroughly searched each day for lice, and the eggs on the clothing were examined for hatching. The number of surviving lice, including the nymphs hatching from the eggs, was recorded.

Further to simulate conditions of natural infestation in treatments that had eliminated all the original infestation of lice, the subjects were reinfested, usually with 100 lice, at the end of the second day following treatment. If the treatment was effective against the reinfestation, the subject was required to wear his underwear continuously for 7 days, at which time he was further reinfested with 100 adults and 100 newly hatched nymphs. If the treatment was effective against the reinfestation introduced at the beginning of the second week, the subject was infested at weekly intervals thereafter until the treatment showed evidence of being ineffective. The garments were not washed during these tests.

Impregnated underwear has been tested by the same method. In testing such garments lice were released in the outer garments of the subject, and 6 hours later the impregnated clothing was put on. Observations were made as in the tests with powders.

#### DEVELOPMENT OF A LOUSE-POWDER FORMULA

*Tests of lousicides.*—In the search for a synthetic toxicant for body lice, several hundred compounds were tested by the beaker method, but only about one-fourth of them were found to be lethal. Since most of the materials tested were known to be toxic to one or more species of insects or were related to effective insecticides, it appears that the body louse is highly resistant to insecticides. Of the materials found to be toxic to lice by the beaker test method, only about one-third were lethal as 5.0 per cent powders when tested by the arm-and-leg method. Most of these treatments were completely effective only as fresh applications, and some lice survived when introduced after the treated garments had been worn for a day or longer.



A louse powder containing a synthetic insecticide has recently been recommended (Bushland et al 1944). During the first few months of the work, however, the results with synthetic organic compounds indicated that they could not be expected to furnish an early solution to the problem. Emphasis was then placed on tests of derris and pyrethrum lousicides.

Spencer (1941) found derris powder an effective control for body lice in Canadian troops, and Busvine (1942) also reported success with a powder containing derris. Pyrethrum powder was recommended for use against lice by Simon (1916), was said to be ineffective as a louse treatment by Nuttall (1918), but a fresh powder was claimed to be effective by Symes et al (1942). Little work has been done on the use of extracts of pyrethrum against lice since that of Juillet and Diacono (1925).

Preliminary tests with finely ground pyrethrum flowers (1.25 per cent of pyrethrins) and derris powder (4.8 per cent of rotenone) indicated that neither material was very effective. In six arm-and-leg tests with ground pyrethrum flowers 9 per cent of the insects survived 24 hours' confinement in freshly treated garments, while in eight tests with derris powder an average of 14.5 per cent of the lice survived.

TABLE 1.—*Comparison of effectiveness against body lice of powders containing 1.0 per cent of pyrethrins and 1.0 per cent of rotenone (prepared from extracts) in tests on grossly infested men*

Material	Number of cases	Per cent of lice surviving on indicated days after infestation				
		1 day	2 days	3 days	4 days	5 days
Pyrethrins .....	2	1.6	1.6	0.4	0.4	0.0
Rotenone .....	3	14.5	7.6	3.3	1.2	1.2

Louse powders prepared by depositing extracts of pyrethrum and derris on an inert diluent were much more effective. For example, pyrophyllite powders containing 1.0 per cent of pyrethrins, deposited from an acetone solution of a 20 per cent concentrate, were completely effective as fresh treatments in tests by the arm-and-leg method. The survival of lice introduced after 24 hours averaged only 0.3 per cent, and these survivors were unable to crawl or feed. After the subjects had worn the treated garments for 4 days, in 19 tests, lice were again introduced. When counts were made after 48 hours, an average of 7.2 per cent of the lice survived.

In 25 tests with 1.0 per cent rotenone powders prepared with derris extract, in which lice were introduced in freshly treated garments, the 24-hour survival averaged 11.5 per cent. Garments were reinfested after 4 days' wearing in 14 cases, and only 7.3 per cent of the lice survived 48 hours after infestation.

These studies indicated that powders containing pyrethrins killed lice more quickly than those made with rotenone (hence the difference in the 24-hour records on fresh treatments), but that, when sufficient time was allowed for rotenone to produce its toxic effects, there was little difference in the results with the two toxicants.

Typical results, on grossly infested men, of powders containing 1.0 per cent of pyrethrins or rotenone prepared by depositing extracts on an inert diluent are shown in Table 1.

These results substantiate those of the arm-and-leg tests. The slow action of rotenone compared with the pyrethrins is particularly evident. Furthermore, rote-

none-containing powders caused severe dermatitis in the scrotal region in about two-thirds of the subjects. It was therefore necessary to discontinue tests of rotenone powders on grossly infested men. No skin irritation has been noted following the use of pyrethrum on the subjects treated at this laboratory.

Because of these two factors—the more rapid kill of powders prepared from pyrethrum extracts and the dermatitis resulting from rotenone powders—the pyrethrum preparations appeared definitely superior to those containing rotenone. As already mentioned, the synthetic organic compounds tested during the early part of the work failed to give extended protection from reinfestation. Since the armed forces were in need of a louse powder for immediate use, pyrethrins appeared most likely to furnish an early solution to the problem of effective treatment.

*Tests of synergists for the pyrethrins.*—As the supply of pyrethrum flowers was limited, the inclusion of a synergist in the louse-powder formula to increase the insecticidal efficacy of the pyrethrins was practically essential. Over 200 materials, chiefly synthetic organic compounds, were tested by the arm-and-leg method as synergists for pyrethrum in pyrophyllite powders.

Very striking results were obtained with several of the compounds. It became evident that many synergists when used with pyrethrum were unusually effective against body lice. One of these materials, N-isobutylundecylenamide (IN-930), was outstanding in its performance. Alone as a 2.0 per cent powder IN-930 was nontoxic to lice. The combination of 2.0 per cent of IN-930 and 0.01 per cent of pyrethrins gave complete kill in 24 hours, while 2.0 per cent of this synergist and 0.005 per cent of pyrethrins killed almost all the lice within 24 hours. Since the mortality obtained at these concentrations was equivalent to that obtained with 1.0 and 0.5 per cent of pyrethrins alone, the toxicity of pyrethrins to lice was increased approximately 100 times by the addition of 2.0 per cent of IN-930. This observation is of great importance, since this synergist only doubles the toxicity of pyrethrins in fly sprays. Subsequent to the recommendation of the pyrethrum louse powder, several compounds have been found that approach IN-930 in increasing the initial kill of lice by the pyrethrins.

The duration of effectiveness of pyrethrum treatments was not prolonged by synergists to the same extent that the initial toxicity was increased. Very few of the synergists had long-lasting effects. Of these, IN-930 was the best. In tests by the arm-and-leg method powders containing 0.2 per cent of pyrethrins and 2.0 per cent of IN-930 were lethal to lice introduced after the garments had been worn for at least 8 days. Because of these results and since it was readily available, IN-930 was adopted as the synergist for inclusion in the louse-powder formula.

*Tests with pyrethrum antioxidants.*—Because pyrethrum powders deteriorate on storage, tests were made with powders containing pyrethrum extract and several common antioxidants. When exposed to air in a thin layer for almost 3 months, pyrethrum powders containing certain antioxidants showed no deterioration, whereas a control powder containing no antioxidant failed to kill lice within 44 days. Of the effective antioxidants, Phenol S (essentially isopropyl cresols obtained as a by-product of thymol manufacture) was selected for use in the louse powder. In subsequent storage tests with the recommended formula, containing 0.25 per cent of Phenol S, the powder did not deteriorate appreciably. When spread in a thin layer and exposed freely to the air for 6 months, the stored powder appeared to be fully

as effective as one freshly prepared. When spread in a watch glass and kept for a month in a constant-temperature oven at 60° C (140° F), a temperature actually encountered in North Africa, the powder showed no evidence of deterioration, but after 2 months at this temperature it was less effective than a freshly prepared control.

*Tests with ovicides.*—The combination of pyrethrum extract, synergist, and anti-oxidant was not ovicidal. As the protection to be expected from the treatment was of shorter duration than the incubation period of body louse eggs, it seemed desirable to incorporate an ovicide in the formula. One of the most effective ovicides found by Eddy (1944) was 2,4-dinitroanisole, which, at relative humidities above 70 per cent, was lethal to louse eggs when applied at a concentration of 5.0 per cent in a dust. However, this compound was almost ineffective against eggs at humidities much below 70 per cent. The ovicidal effect of dinitroanisole was increased by the addition of IN-930, and 2.0 per cent of each in a powder at favorable humidities was sufficient to kill eggs in all stages of incubation up to within at least 1 day of hatching. In addition to possessing ovicidal properties, 2,4-dinitroanisole was found to be an effective lousicide.

#### THE MYL FORMULA

As a result of the foregoing studies, the following formula for a louse powder was developed:

	<i>Per cent</i>
Pyrethrins (from a 20% concentrate) .....	0.2
IN-930 .....	2.0
2,4-dinitroanisole .....	2.0
Phenol S .....	0.25
Pyrophyllite to make 100 per cent	

After extensive tests this powder, which is designated as "MYL," was recommended to the armed forces in September 1942. It has been approved as safe for continued use on human skin.<sup>5</sup>

In laboratory preparation the active ingredients were dissolved in acetone and added to the pyrophyllite. The solvent was then allowed to evaporate and the powder ground with a spatula on a glass plate. Commercial lots were prepared by dissolving the toxicant, synergist, and antioxidant in alcohol denatured with acetone and spraying this solution into the powder. The ovicide in a finely ground state was mechanically mixed with this powder. Frequent biological tests of random samples of commercial preparations purchased by the Army and Navy indicated that this method was generally satisfactory.

In the period since the MYL formula was developed, it has been tested repeatedly by the arm-and-leg method and on grossly infested subjects. At least a week's protection has been obtained under all the test conditions. During warm weather the MYL treatment in arm-and-leg tests usually gave complete protection for 8 days. In cool weather the treatment frequently was effective for 10 or 12 days. In tests with whole suits of underwear on grossly infested men the powder usually killed all lice introduced at the beginning of the second week's wearing, but failed to give complete control of lice introduced after this.

<sup>5</sup> Pharmacological tests of this and other formulas were made by H. O. Calvery, of the U. S. Food and Drug Administration, under a transfer of funds from the Office of Scientific Research and Development.

The MYL powder has been compared with a number of other louse powders and some of the results given below are typical. In table 2 are shown results obtained with MYL, with and without 2,4-dinitroanisole, and with AL-63<sup>6</sup> and 10 per cent lauryl thiocyanate. The last two have been in use in the British Army.

TABLE 2.—Comparison of effectiveness against body lice of MYL and other powders in tests by the arm-and-leg method

Treatment	Percentage survival after 48 hours when lice were introduced after treated garments had been worn		
	4 days	6 days	8 days
MYL .....	0	0.0	0
MYL without 2,4-dinitroanisole .....	0	0.0	4
AL-63 .....	8	—	—
10% lauryl thiocyanate* .....	4	9.3	—

\* Not tested simultaneously with the other treatments.

Although the AL-63 was effective as a fresh treatment, it had begun to fail by the fourth day and was discontinued to avoid annoyance to the test subjects. The 10 per cent lauryl thiocyanate was also effective as a fresh treatment, but allowed an appreciable number of lice to survive after 3 days' wearing.

Table 3 gives the comparative results obtained with MYL, with and without 2,4-dinitroanisole, and AL-63 in tests on grossly infested men.

TABLE 3.—Comparison of effectiveness against body lice of MYL and other powders in tests on grossly infested men

Treatment	Number of subjects	Number of lice present when treated*	Number of lice surviving on indicated days after infestation—									
			1 day		2 days		3 days		4 days		5 days	
			A.†	N.	A.	N.	A.	N.	A.	N.	A.	N.
MYL .....	4	1,539	0	0	0‡	0	3	0	0	0	0	0
MYL without 2,4-dinitroanisole ...	5	1,837	4	0	0‡	0	14	24	0	15	1	37
AL-63 .....	5	758	15	0	5	0	1	2	0	43	0	50

\* Several thousand eggs were also introduced with the initial infestation of adult lice.

† A = adults; N = nymphs.

‡ At this time 400 to 500 adult lice were added.

It is interesting to note that a number of newly hatched nymphs survived 3 days' wearing of the garment treated with MYL without the ovicide, whereas with the complete MYL formula no nymphs had appeared after 5 days. The AL-63 powder also showed increasing numbers of nymphs. This powder caused dermatitis in the scrotal region of three out of the five subjects.

*Speed of knockdown, ability to feed, and kill of lice exposed to MYL.*—The time required for a treatment to kill lice or to prevent further feeding is considered highly important from the standpoint of typhus control. Tests were conducted to determine the speed of action of the MYL formula. Hungry lice exposed to MYL for only 10 minutes were unable to feed, which would in some instances be equivalent to their death. All the lice were knocked down or immobilized in 15 minutes' exposure to this powder. With freshly fed lice, about 50 per cent were killed in 6 hours and 100 per cent mortality occurred after 10 hours' exposure. In comparison with insecticides in general, the action of MYL on lice is considered rapid.

<sup>6</sup> A British product containing derris resins and other materials.



*Use against crab lice and head lice.*—The MYL powder has been used to treat over 100 natural infestations of crab lice at this laboratory. These cases were obtained through the cooperation of the Army Air Forces School of Applied Tactics at Orlando. The powder has also been used for crab louse control by Army and Navy medical officers at Camp Claiborne, La., Patterson Field, Ohio, and the Naval Air Station, Corpus Christi, Tex.

When all hairy portions of the body were dusted with the powder and the treatment was not removed by washing until the following day, all motile forms of the louse were killed. Such a treatment was not sufficiently ovicidal to insure destruction of all the eggs, although cooperating medical officers reported many cases of eradication with a single treatment. It is recommended that a second treatment be applied after 8 to 10 days to kill the nymphs hatching from any eggs that might survive. In treating infestations of crab lice it is essential that all hairy areas be dusted, since these lice are often present on portions of the body sparsely covered with hair.

MYL was used in the treatment of five cases of head lice. Three of the patients had their hair washed 1, 2, and 4 days after treatment. All the motile forms were destroyed, but a few newly emerged nymphs were found on subsequent examinations. In the two cases in which the hair was not washed for at least 10 days, the individuals remained free of lice.

These results appear to indicate that the formula could be employed successfully for the treatment of head lice. A second application should be made 10 to 14 days after the first.

The active ingredients of the MYL powder have been also applied in a liquid formula and found effective at lower concentrations than when employed in the powder treatment.

*Use against other pests.*—The MYL powder has been used successfully against ants, fleas, bedbugs, and other troublesome insects encountered by troops and is highly regarded in active theaters of war as a general purpose insecticide. The powder has been the standard issue for individual soldiers, and reports indicate that while other items of equipment may be thrown away the 2-ounce cans of MYL are retained.

#### SUMMARY

Test methods used in the laboratory evaluation of insecticides for the body louse are described. These include procedures for beaker tests, arm-and-leg tests, ovicide tests, and tests on grossly infested men.

The development of an effective louse powder for use by the armed forces is described. The louse powder, the MYL formula, consists of 0.2 per cent of pyrethrins as a toxicant, 2.0 per cent of IN-930 as a synergist, 0.25 per cent of Phenol S as an antioxidant, and 2.0 per cent of 2,4-dinitroanisole as an ovicide, with pyrophyllite as an inert diluent. It was established that this powder does not readily deteriorate even under unfavorable storage conditions.

Laboratory comparisons by the arm-and-leg method and tests on grossly infested men showed the MYL formula to be a superior louse powder. This powder, uniformly applied over the entire inner surface of winter underwear worn by heavily infested men at a dosage of 30 grams per suit, killed all body lice present at the time of treatment. It also killed all eggs with which it came in contact, and gave complete protection against introduced lice for at least a week.

The MYL powder was also found to be an effective control for head lice and crab lice. It is also being employed successfully by troops in war theaters against other important insects, such as fleas, bedbugs, and ants.

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# AMERICAN SOCIETY OF PARASITOLOGISTS

## THIRTY-THIRD COUNCIL MEETING, CLEVELAND, OHIO

SEPTEMBER 11, 1944

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. H. E. Ewing, President of the Society, at 3:00 PM, September 11, 1944, in the Hotel Statler, Cleveland, Ohio. Past-presidents J. E. Ackert and G. R. La Rue and the following members of the Council were present: D. L. Augustine, R. M. Cable, J. T. Culbertson, H. E. Ewing, C. G. Huff, G. F. Otto, H. W. Stunkard, and W. H. Wright.

### I. REPORTS OF OFFICERS

1. *Secretary (J. T. Culbertson)*: As of August 15, 1944, there were 554 persons on the membership roll of the Society of which 493 lived within and 61 lived outside the continental United States. Of the total number on the roll, 448 were members in good standing and 106 were delinquent for one (50), two (31), or three (25) years. Of those in good standing 408 lived within and 40 lived outside the continental United States. Fifty new members were elected to date during 1944, of which 46 were domestic and 4 foreign. The death of one member occurred during the year: Father James J. Regan, St. Mary's Seminary, Baltimore, Maryland, on August 5, 1944.

Upon motion, the Secretary's report was accepted and placed on file.

2. *Treasurer (G. F. Otto)*: On December 6, 1943, there was a balance on hand of \$2957.16. Collections to September 1, 1944, from all sources during the year, including dues from members or applicants for membership, non-member subscriptions to JOURNAL, sales of back numbers of JOURNAL, copies of index, portraits, and advertisements, as well as interest on savings account, amounted to \$4020.28. Total available funds, therefore, equalled \$6977.44. Expenditures to September 1, 1944, were, for printing three numbers of JOURNAL, \$1629.36; for office of Chairman of Editorial Committee, \$93.16; for office of Secretary, \$184.77; for office of Treasurer, \$334.75, these amounting altogether to \$2242.04. As of September 1, 1944, there remains, therefore, a cash balance of \$4735.40.

Upon motion, the Treasurer's report was accepted and placed on file, audit to be made at fiscal year-end.

### II. REPORT OF COMMITTEES

#### 1. *Custodian of Princeton Secretarial Fund (N. R. Stoll)*:

	Sept. 7, 1944	Year ending Dec. 3, 1943
A. Total liquid assets .....	\$1023.45	\$998.55
B. Total, last previous report .....	998.55	475.79
Gain in interim .....	\$ 24.90	\$522.76

Upon motion, report of Custodian was accepted and placed on file, audit to be made at end of fiscal year.

2. *Chairman of Editorial Committee (H. W. Stunkard)*: Shortage of submitted manuscripts and labor shortage in the printer's office have been responsible for the tardy appearance of the 1944 numbers of the JOURNAL. It is believed now, however, that enough material will be available to issue all six regular numbers of the JOURNAL plus Supplement to the August issue. It is hoped that beginning with the October issue, numbers will appear on time.

Upon motion, the report was accepted.

### III. REPORT OF REPRESENTATIVES

1. *Representatives to Council of A.A.A.S.*: No report was received from the representatives to the Council of A.A.A.S.

2. *Representatives to Council of U.A.B.S. (A. C. Walton)*: The Union of American Biological Societies was endeavoring to create an organization comprising all aspects of biology and able, therefore, to represent nationally all the biological sciences. The only publication which at the moment the Union proposed to sponsor was Biological Abstracts. It was hoped that every participating Society would contribute generously, a fixed fee being suggested, this based roughly on Society membership.

Following motion, Dr. Walton's report was accepted. Council then voted against contributing to the Union at this time.

## IV. NEW BUSINESS

1. *New Members*: Council approved membership in the Society of nine applicants: Clyde Carlton Blackburn, Madison College, Tennessee; Hampton L. Carson, Zoology Department, Washington University, St. Louis, Missouri; Robert Greenwood Grocott, Board of Health Laboratory, Ancon, Canal Zone; Lt. Herman E. Marholin, 40th Malaria Survey Unit, A.P.O. 322, c/o P.M., San Francisco, California; Eleanor Mae Neumayer, Zoological Division, U. S. Bureau of Animal Industry, Beltsville, Maryland; Harry E. Ohl, 15th Medical General Laboratory, A.P.O. 534, c/o P.M., New York, N. Y.; Irwin H. Roberts, U. S. Bureau of Animal Industry, Albuquerque, New Mexico; William F. Simpson, Department of Biology, Catholic University of America, Washington, D. C.; Edward D. Wagner, Zoology Department, State College of Washington, Pullman, Washington.

2. *Management of Journal*: It was recommended by Council that Treasurer, Custodian of JOURNAL stock, and Chairman of Editorial Committee endeavor to build up the reserve of back numbers of the JOURNAL and extend Society funds for this purpose as necessary. Dr. Ackert recommended the reprinting of some JOURNAL issues which now are short. The Council recommended a small increase in the number of exchanges, at the discretion of the Chairman of the Editorial Committee.

The establishment of a monograph series for the JOURNAL was discussed, but no decision was reached on the matter. It was agreed that the present was an unfavorable time for initiating such a series. Council recommended that the Editorial Committee use its own discretion on charges to authors, varying its basic plan with variations in number of submitted manuscripts. The regular inclusion of an application form for membership in the Society was discussed. Earlier, Council had recommended inclusion.

3. *Place for Meeting in 1945*: No decision was reached as to the place for meeting in 1945.

4. *Nomination or Election of Officers, Appointments, etc.*

- a. The following persons were nominated by Council to the Society offices: A. C. Chandler, for President for the year 1945; D. L. Augustine, for Vice-President for the year 1945; R. M. Stabler, for Treasurer for two years beginning 1945; G. R. Coatney and G. F. Otto, for Councillors-at-Large for four years beginning 1945.
- b. The following persons were elected by Council to the Editorial Board of the JOURNAL OF PARASITOLOGY: C. W. Rees, L. A. Spindler, and W. L. Jellison.
- c. No appointments to standing committees were made, the President delaying these until later in the year.

Council voted its thanks to the Society Officers and Representatives and adjourned at 6:15 PM.

Respectfully submitted,

J. T. CULBERTSON, *Secretary*.

## AMERICAN SOCIETY OF PARASITOLOGISTS

NINETEENTH ANNUAL MEETING, CLEVELAND, OHIO\*

SEPTEMBER 11 AND 12, 1944

*Minutes of General Business Meeting*

The general business meeting of the Society was held at the Academy of Medicine, Cleveland, Ohio, on September 12, 1944. Twenty-five members of the Society were present. The meeting was called to order at 12:15 PM by President H. E. Ewing.

The reports of the Secretary, Treasurer, and Chairman of the Editorial Committee were presented to the Society, as was the report of the representatives to the Council of the Union of American Biological Societies.

The Secretary presented to the Society the names of persons nominated by Council for the Society offices. There were no further nominations and upon motion, duly seconded and carried, the Secretary was instructed to cast one ballot for the nominations as presented. Accordingly, the following persons were elected to the Society offices: President for one year, A. C. Chandler; Vice-President for one year, D. L. Augustine; Treasurer for two years, R. M. Stabler; Councilors-at-Large for four years, G. R. Coatney and G. F. Otto.

A vote of thanks was extended to Dr. Paul D. Harwood for his services as local representative in making arrangements for the Cleveland meeting.

At 1:15 PM, it was voted that the Society adjourn to convene again at a place and time selected by Council.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary*.

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\* Prior to the scheduled meeting, the Program Committee found it necessary to comply with a directive from the Office of Defense Transportation urging cancellation of all meetings not directly related to the war. Accordingly, the general program of the Society was cancelled and only a Symposium on "Parasitology in Relation to the War" was held in Cleveland. Abstracts of all papers submitted for the meeting appeared in the JOURNAL OF PARASITOLOGY, August Supplement, 1944.

# INDEX FOR VOLUME 30, NOS. 1-6

ADDIS, C. T. AND ASA C. CHANDLER. Studies on the vitamin requirement of tapeworms . . . . .	229
<i>Adelina deronis</i> , coccidian parasite, factors influencing the course of infection . . . . .	162
<i>Alloglossidium corti</i> , development of sporocyst stages . . . . .	37
<i>Amblyomma americanum</i> , occurrence in Minnesota and Ohio . . . . .	200
<i>Amblyomma</i> (Ixodidae) in the United States . . . . .	77
AMEEL, DONALD J. The life history of <i>Nudacotyle novicia</i> Barker, 1916 (Trematoda: Noto- cotylidae) . . . . .	257
AMEEL, DONALD J. (see Cort and Ameel) . . . . .	37
AMEEL, DONALD J. (see Cort, Ameel and Olivier) . . . . .	1
AMERICAN SOCIETY OF PARASITOLOGISTS:	
Minutes, 19th annual meeting, Cleveland, Ohio . . . . .	392
Minutes, 32nd council meeting . . . . .	203
Minutes, 33rd council meeting, Cleveland, Ohio . . . . .	390
Notice of partial cancellation of Cleveland program . . . . .	275
Preliminary announcement of the 19th annual meeting . . . . .	130
Second report of the committee on terminology of strains of avian malaria . . . . .	206
War-time activities of . . . . .	195
ANDERSON, DORCAS J. Studies on <i>Cercaria ssidati</i> sp. nov., a furcocercous cercaria of the Vivax type . . . . .	264
ARMER, SISTER JOSEPH MARIE. Influence of the diet of Blattidae on some of their intestinal Protozoa . . . . .	131
<i>Ascaridia galli</i> , effect of nicotine-bentonite and phenothiazine on . . . . .	143
<i>Ascaris lumbricoides</i> , effect of radiation on the eggs of . . . . .	26
AUGUSTSON, GUSTAF F. A new mouse flea, <i>Pleochaetoides bullisi</i> n. gen., n. sp., from Texas . . . . .	366
AUGUSTSON, GUSTAF F. The flea genus <i>Thrassis</i> and sylvatic plague, with the description of <i>T. brennani</i> n. sp. . . . .	237
<i>Blattella germanica</i> , cockroach, host of new parasitid mite . . . . .	181
Blattidae, influence of diet on their intestinal Protozoa . . . . .	131
Blood fluke, new cercaria of, from marine pelecypod . . . . .	191
BOARDMAN, EDWARD T. Methods for collecting ticks for study and delineation . . . . .	57
BRACKETT, STERLING (see Cort, Brackett and Olivier) . . . . .	309
BRADY, FREDERICK J. AND ALFRED H. LAWTON. A new method for quantitative estimation of microfilariae in blood samples . . . . .	34
BRAND, THEODOR VON AND W. F. SIMPSON. Physiological observations upon a larval <i>Eustrongylides</i> . VII. Studies upon survival and metabolism in sterile surroundings . . . . .	121
<i>Brevoortia tyrannus</i> , host of <i>Eimeria brevoortiana</i> n. sp. . . . .	60
BUSHLAND, R. C., L. C. McALISTER, JR., G. W. EDDY, HOWARD A. JONES AND E. F. KNIP- LING. Development of a powder treatment for the control of lice attacking man . . . . .	377
<i>Cercaria marilli</i> Ameel 1939, larval stage of <i>Nudacotyle novicia</i> . . . . .	257
<i>Cercaria talboti</i> , development of sporocyst stages . . . . .	37
Cestodes, vitamin requirements of . . . . .	229
Chagas' disease, feeding habits of hemipterid vectors . . . . .	197
CHANDLER, ASA C. A new species of <i>Mesocestoides</i> , <i>M. kirbyi</i> , from <i>Canis latrans</i> . . . . .	273
CHANDLER, ASA C. (see Addis and Chandler) . . . . .	229
CHEATUM, E. L. (see Goble and Cheatum) . . . . .	119
CHEN, H. T. <i>Spelotrema pseudogonotyla</i> n. sp. (Trematoda: Microphallidae) from Hong- kong . . . . .	159
Chigger mites, morphology, life history, distribution, taxonomy and relation to disease . . . . .	339
Coccidian infection of <i>Dero limosa</i> , factors influencing . . . . .	162
COLE, C. L. (see Hawkins, Cole and Kline) . . . . .	373
COOLEY, R. A. <i>Ixodes osarkus</i> n. sp., and <i>Ornithodoros aquilae</i> n. sp. with notes on <i>O. talaje</i> and <i>O. kelleyi</i> (Ixodoidea) . . . . .	287
COOLEY, R. A. AND GLEN M. KOHLS. The genus <i>Amblyomma</i> (Ixodidae) in the United States . . . . .	77
CORT, W. W. AND D. J. AMEEL. Further studies on the development of the sporocyst stages of plagiorchiid trematodes . . . . .	37
CORT, W. W., D. J. AMEEL AND LOUIS OLIVIER. An experimental study of the development of <i>Schistosomatum douthitti</i> (Cort, 1914) in its intermediate host . . . . .	1



CORT, W. W., STERLING BRACKETT AND LOUIS OLIVIER. Lymnaeid snails as second intermediate hosts of the strigeid trematode, <i>Cotylurus flabelliformis</i> (Faust, 1917) .....	309
<i>Cotylurus flabelliformis</i> , lymnaeid snails as second intermediate hosts .....	309
<i>Culex molestus</i> , insect host of filariid nematode of frogs .....	245
DAVIS, HELEN EDITH. <i>Cittotaenia sandgroundi</i> , a new anoplocephalid cestode from a Javanese tree duck .....	241
DENTON, J. FRED. Studies on the life history of <i>Eurytrema procyonis</i> Denton, 1942 .....	277
Development of <i>Hymenolepis</i> in mice, effect of concurrent infection with <i>Nippostrongylus</i> ...	18
Development of <i>Schistosomatium douthitti</i> in its intermediate host .....	1
Development of the sporocyst stages of the plagiorchiid trematodes .....	37
<i>Dientamoeba fragilis</i> , morphology, life history, diagnosis and host-relations .....	322
Diet of Blattidae, influence on intestinal Protozoa .....	131
DOETSCHMAN, WILLIS H. Notes on Anoplura infesting marine carnivores .....	200
DOLL, R. B. (see Hauschka and Doll) .....	198
DONALDSON, ALAN W. (see Larsh and Donaldson) .....	18
EDDY, G. W. (see Bushland, McAlister, Eddy, Jones and Knipling) .....	377
Effect of concurrent infection with <i>Nippostrongylus</i> on the development of <i>Hymenolepis</i> in mice .....	18
<i>Eimeria tenella</i> , the respiration of .....	295
<i>Embadomonas intestinalis</i> , incidence in food handlers and diarrhetic patients .....	34
<i>Enterobius vermicularis</i> , effect of radiation on the eggs .....	26
<i>Eurytrema procyonis</i> , the life history of .....	277
<i>Eustrongylides ignotus</i> , survival and metabolism of larvae in sterile surroundings .....	121
EWING, H. E. The trombiculid mites (chigger mites) and their relation to disease .....	339
Exoerythrocytic stages of <i>Plasmodium durac</i> .....	177
FELSENFELD, OSCAR. (see Young and Felsenfeld) .....	34
Filariae of amphibians .....	245
FISCHTHAL, JACOB H. Observations on a sporozoan parasite of the eelpout, <i>Zoarces anguillaris</i> , with an evaluation of candling methods for its detection .....	35
Flea, new genus and species from mouse in Texas .....	366
<i>Foleyella duboisi</i> , morphology and life history of .....	245
FUJII, HAROLD. Three monogenetic trematodes from marine fishes .....	153
Gapeworm, <i>Syngamus trachea</i> , acquired resistance to .....	68
GATES, DORIS B. <i>Xenopsylla cheopis</i> in Lincoln, Nebraska .....	202
GERICHTER, CH. (see Witenberg and Gerichter) .....	245
<i>Giardia sanguinis</i> , host of .....	202
GOBLE, FRANS C. AND E. L. CHEATUM. Notes on the lungworms of North American Leporidae .....	119
GUTHRIE, JAMES E. (see Harwood and Guthrie) .....	143
GUTHRIE, JAMES E. (see Harwood and Guthrie) .....	197
HARDCASTLE, A. B. <i>Eimeria brevoortiana</i> , a new sporozoan parasite from Menhaden ( <i>Brevoortia tyrannus</i> ), with observations on its life history .....	60
HARWOOD, PAUL D. AND JAMES E. GUTHRIE. The effect of nicotine-bentonite and of certain physical states upon the efficacy of phenothiazine against nematodes in fowls .....	143
HARWOOD, PAUL D. AND JAMES E. GUTHRIE. The effect of prickly-ash bark upon the efficacy of phenothiazine against nematodes in fowls .....	197
HAUSCHKA, THEODORE. Season, nutrition, immunity, drugs and X-ray as factors influencing the course of a coccidian infection .....	162
HAUSCHKA, T. S. AND R. B. DOLL. <i>Paraglaucoma sp.</i> , a facultative parasite of coelenterates .....	198
HAWKINS, PHILIP A., C. L. COLE AND E. E. KLINE. Studies on sheep pastures. IV. Survival of sheep nematodes on pasture during the fall months .....	373
HERMAN, CARLTON M. Notes on the pupal development of <i>Stilbometopa impressa</i> (Diptera: Hippoboscidae) .....	112
HERRICK, C. A. (see Smith and Herrick) .....	295
<i>Heterakis gallinae</i> , effect of nicotine-bentonite and phenothiazine on .....	143
HOGUE, M. J. <i>Trichomonas vaginalis</i> in the fluid of the vagina .....	34

HOLLAENDER, ALEXANDER. (see Jones and Hollaender) .....	26
Host relations of strigeid trematode, <i>Cotylurus flabelliformis</i> .....	309
<i>Hymenolepis</i> in mice, effect of infection with <i>Nippostrongylus</i> on the development of .....	18
<i>Hymenolepis nana</i> var. <i>fraterna</i> , development in different mice .....	21
Irradiation, effect of, on resistance of rats to infection by <i>Trypanosoma lewisi</i> .....	209
Ixodidae, <i>Amblyomma</i> in the United States .....	77
Ixodoidea, new species of <i>Ixodes</i> and <i>Ornithodoros</i> .....	287
JAMES, MAURICE T. Two erroneous records in American literature of the causative agents of myiasis .....	273
JONES, HOWARD A. (see Bushland, McAlister, Eddy, Jones and Knipling) .....	377
JONES, MYRNA F. AND ALEXANDER HOLLAENDER. Effect of long ultraviolet and near visible radiation on the eggs of the nematodes <i>Enterobius vermicularis</i> and <i>Ascaris lumbricoides</i> .....	26
KEEGAN, HUGH L. On a new genus and species of parasitid mite .....	181
KLINE, E. E. (see Hawkins, Cole and Kline) .....	373
KNIPLING, E. F. (see Bushland, McAlister, Eddy, Jones and Knipling) .....	377
KOHL, GLEN M. (see Cooley and Kohls) .....	77
LARSH, JOHN E., JR. Comparative studies on a mouse strain of <i>Hymenolepis nana</i> var. <i>fraterna</i> , in different species and varieties of mice .....	21
LARSH, JOHN E., JR. AND ALAN W. DONALDSON. The effect of concurrent infection with <i>Nippostrongylus</i> on the development of <i>Hymenolepis</i> in mice .....	18
LAWTON, ALFRED H. (see Brady and Lawton) .....	34
<i>Leishmania chagasi</i> , identical with <i>L. donovani</i> .....	303
Leishmaniasis, etiological agent of American visceral type .....	303
Leporidae, lungworms of North American .....	119
Lice attacking man, powder treatment for control of .....	377
Lice, infesting marine carnivores .....	200
Life history of <i>Eimeria brevoortiana</i> , observations on .....	60
Life history of <i>Eurytrema procyonis</i> .....	277
Life history of <i>Foleyella duboisi</i> (Geddoelst, 1916) .....	245
Life history of <i>Nudacotyle novicia</i> .....	257
Lungworms of North American Leporidae .....	119
<i>Macroderoides typicus</i> , development of sporocyst stages .....	37
MARAVENTANO, L. W. (see Nigrelli and Maraventano) .....	184
Marine fishes, new monogenetic trematodes from .....	153
MARTIN, W. E. <i>Cercaria solemyae</i> n. sp., probably a blood fluke, from the marine pelecypod, <i>Solemya velum</i> .....	191
McALISTER, L. S., JR. (see Bushland, McAlister, Eddy, Jones and Knipling) .....	377
MELONEY, HENRY E. War-time activities of members of the American Society of Parasitologists .....	195
<i>Mesodon thyroidus</i> , snail host of <i>Eurytrema procyonis</i> .....	277
Microfilariæ, a new method for quantitative estimation of, in blood samples .....	34
Mole cricket, <i>Gryllotalpa hexadactyla</i> , host of new species of <i>Retortamonas</i> .....	173
Monogenetic trematodes from marine fishes .....	153
Myiasis, erroneous records of causative agents .....	273
NAIMAN, DOROTHY NEUHOF. Effect of x-irradiation of rats upon their resistance to <i>Trypanosoma lewisi</i> .....	209
<i>Necator americanus</i> , a mounting method for .....	201
Nematodes in fowls, effects of prickly-ash bark and phenothiazine against .....	197
Nematodes of sheep, survival on pasture during fall months .....	373
<i>Neoechinorhynchus emydis</i> , responses to various solutions .....	369
New genera (indicated *) and new species (volume 30, 1944):	
<i>Eimeria brevoortiana</i> Hardcastle .....	60
<i>Hexastoma macracanthum</i> Fujii .....	153
<i>Cyclocotyla hysteroncha</i> Fujii .....	154
* <i>Microcotyloides</i> Fujii, type <i>M. incisa</i> (Linton, 1910) .....	155
<i>Paramphistomum microbothroides</i> Price.....Supplement, Abstract	13

<i>Spelotrema pseudogonotyla</i> Chen .....	159
<i>Retortamonas wenrichi</i> Stabler .....	173
* <i>Transversotrema</i> Witenberg .....	179
* <i>Blattisocius</i> Keegan .....	181
<i>Diplostomulum xenopi</i> .....	184
<i>Cercaria solemyae</i> .....	191
<i>Thrassis brennani</i> .....	237
<i>Cittotaenia sandgroundi</i> .....	241
<i>Cercaria ssidati</i> .....	264
<i>Pseudonymus brachycercus</i> .....	269
<i>Pseudonymus leptocercus</i> .....	269
<i>Mesocestoides kirbyi</i> .....	273
* <i>Barroella</i> Zeff .....	275
<i>Ixodes ogarkus</i> .....	287
<i>Ornithodoros aquilae</i> .....	287
* <i>Pleochaetoides bullisi</i> .....	366
Nicotine-bentonite, its effect on efficacy of phenothiazine .....	143
NIGRELLI, ROSS F. AND L. W. MARAVENTANO. Pericarditis in <i>Xenopus laevis</i> caused by <i>Diplostomulum xenopi</i> sp. nov., a larval strigeid .....	184
<i>Nippostrongylus</i> , effect of infections with, on the development of <i>Hymenolepis</i> in mice .....	18
<i>Nudacotyle novicia</i> Barker, life history of .....	257
<i>Nyctotherus ovalis</i> , effect of diet of host on .....	131
OLIVIER, LOUIS. Acquired resistance in chickens, turkeys, and ring-necked pheasants to the gapeworm, <i>Syngamus trachea</i> .....	69
OLIVIER, LOUIS. (see Cort, Ameel and Olivier) .....	1
OLIVIER, LOUIS. (see Cort, Brackett and Olivier) .....	309
<i>Paraglaucoma</i> sp., a facultative parasite of <i>Hydra</i> .....	198
Parasitid mite, a new genus and species from cockroach .....	181
Pericarditis in <i>Xenopus laevis</i> caused by larval strigeid .....	184
Phenothiazine, effect against nematodes of fowls .....	197
Phenothiazine, effect of nicotine-bentonite on its efficacy against fowl nematodes .....	143
<i>Phlebotomus limai</i> , occurrence in the United States .....	274
Physiological observations upon a larval <i>Eustrongylides</i> .....	121
Plagiorchiid trematodes, development of the sporocyst stages of .....	37
<i>Plagiorchis micracanthos</i> , development of sporocyst stages .....	37
<i>Plagiorchis muris</i> , development of sporocyst stages .....	37
<i>Plagiorchis proximus</i> , development of sporocyst stages .....	37
<i>Plasmodium durae</i> , exoerythrocytic stages of .....	177
<i>Polymorphus</i> sp., a hermit crab as intermediate host of .....	201
<i>Pomatiopsis lapidaria</i> , intermediate host of <i>Nudacotyle novicia</i> .....	257
Powder treatment for control of lice attacking man .....	377
<i>Protostrongylus boughtoni</i> in North American Leporidae .....	119
Pupal development of <i>Stilbometopa impressa</i> .....	112
REINHARD, EDWARD G. A hermit crab as intermediate host of <i>Polymorphus</i> (Acanth.) ....	201
Resistance of chickens, turkeys and pheasants to gapeworm .....	69
Resistance of irradiated rats to infections by <i>Trypanosoma lewisi</i> .....	209
Respiration of protozoan parasite, <i>Eimeria tenella</i> .....	295
RILEY, WILLIAM A. The occurrence of <i>Amblyomma americanum</i> in Minnesota and Ohio ..	200
ROSS, ELIZABETH L. (see Van Cleave and Ross) .....	369
ROZEBOOM, L. E. <i>Phlebotomus limai</i> Fonseca in the United States (Diptera: Psychodidae). ..	274
<i>Schistosomatum douthitti</i> (Cort, 1914), development in intermediate host .....	1
SENEKJIE, HARRY A. American visceral leishmaniasis—the etiological agent .....	303
Sheep nematodes, survival on pastures .....	373
SIMPSON, MYRON L. Exoerythrocytic stages of <i>Plasmodium durae</i> .....	177
SIMPSON, W. F. (see Brand and Simpson) .....	121
SMITH, B. F. AND C. A. HERRICK. The respiration of the protozoan parasite, <i>Eimeria</i> <i>tenella</i> .....	295
Sporozoan parasite, <i>Eimeria brevoortiana</i> , from Menhaden ( <i>Brevoortia tyrannus</i> ) .....	60
Sporozoan parasite of the eelpout, <i>Zoarces anguillaris</i> .....	35

STABLER, ROBERT M. A new species of <i>Retortamonas</i> (Protozoa) from the common mole cricket, <i>Gryllotalpa hexadactyla</i> .....	173
STABLER, ROBERT M. <i>Giardia sanguinis</i> (Gonder, 1911) not from a falcon .....	202
<i>Stilbometopa impressa</i> (Diptera: Hippoboscidae) pupal development of .....	112
SULLIVAN, THELMA D. Viability of <i>Trypanosoma cruzi</i> in citrated blood stored at room temperature .....	200
Sylvatic plague, relation of fleas of genus <i>Thrassis</i> to .....	237
<i>Syngamus trachea</i> , gapeworm, acquired resistance to .....	68
<i>Synthetocaulus leporis</i> in North American Leporidae .....	119
<i>Thrassis</i> , a review of the genus, relation to sylvatic plague, description of a new species ....	237
Ticks, methods for collecting .....	57
TODD, A. C. Two new nematodes from the aquatic beetle, <i>Hydrous triangularis</i> (Say) ....	269
Transversotreminae n. subfam. (Trematoda) .....	180
<i>Triatoma</i> spp., feeding habits of .....	197
<i>Trichomonas vaginalis</i> in the fluid of the vagina .....	34
Trombiculid mites, morphology, life history, taxonomy and relation to disease .....	339
<i>Trypanosoma cruzi</i> , new locality for .....	199
<i>Trypanosoma cruzi</i> , viability of in stored, citrated blood .....	200
<i>Trypanosoma lewisi</i> , effect of radiation of rats on resistance to .....	209
Tsutsugamushi disease, etiology and epidemiology .....	339
Typhus like diseases trombiculid mites .....	339
VAN CLEAVE, HARLEY J. AND ELIZABETH L. ROSS. Physiological responses of <i>Neoechinorhynchus emydis</i> (Acanthocephala) to various solutions .....	369
Vitamin requirements of cestodes .....	229
Vivax group of furcocercous cercariae, a new species and taxonomy of .....	264
WENRICH, D. H. Studies on <i>Dientamoeba fragilis</i> (Protozoa). IV. Further observations, with an outline of present-day knowledge of this species .....	322
WITENBERG, G. <i>Transversotrema haasi</i> , a new fish trematode .....	179
WITENBERG, G. AND CH. GERICHTER. The morphology and life history of <i>Foleyella duboisi</i> with remarks on allied filariids of Amphibia .....	245
WOOD, SHERWIN F. An additional California locality for <i>Trypanosoma cruzi</i> Chagas in the western cone-nosed bug, <i>Triatoma protracta</i> .....	199
WOOD, SHERWIN F. Notes on the feeding of cone-nosed bugs (Hemiptera, Reduviidae) ...	197
<i>Xenopsylla cheopis</i> , in Nebraska .....	202
YETWIN, I. JACQUES. A simple permanent mounting method for <i>Necator americanus</i> .....	201
YOUNG, VIOLA MAE AND OSCAR FELSENFELD. The incidence of <i>Embadomonas intestinalis</i> Wenyon and O'Connor in food handlers and diarrheic patients of mental hospitals .....	34
ZELIFF, C. COURSON. <i>Barroella</i> n. nom. for <i>Kirbyella</i> Zelif, 1930, homonym .....	275
<i>Zoarcis anguillar</i> , the eelpout, a sporozoan parasite of .....	35



# INDEX FOR AUGUST SUPPLEMENT, 1944

(\* refers to abstract number rather than page)

Acanthocephala, physiological responses of <i>Neoechinorhynchus emydis</i> to various solutions .	5*
ACKERT, J. E., DOROTHY S. BRANSON, D. J. AMEEL AND B. B. RIEDEL. Substitution of soy-bean oil meal for animal protein in developing resistance of animals to parasitism . . . .	26*
ACKERT, J. E. AND C. L. WISSEMAN. Studies on effects of helminths on growing chickens .	27*
<i>Adelina deronis</i> , parasite of <i>Dero limosa</i> . . . . .	16*
ALLEN, EVELYN. (see Otto, Allen and Brackett) . . . . .	21*
AMEEL, D. J. (see Ackert, Branson, Ameel and Riedel) . . . . .	26*
AMERICAN SOCIETY OF PARASITOLOGISTS:	
<i>In memoriam</i> . . . . .	22
Members elected 1943-1944 . . . . .	23
Officers . . . . .	19
Program 19th annual meeting, Cleveland, Ohio . . . . .	1
Aryl arsonic acids, effect on experimental coccidiosis infection in chickens . . . . .	3*
<i>Ascaridia galli</i> , effects of host starvation on . . . . .	25*
<i>Australorbis glabratus</i> , elimination of by water flow . . . . .	34*
Author Index . . . . .	4
BOWMAN, GEORGE W. (see Hammond, Davis, Bowman and Simms) . . . . .	4*
BRACKETT, STERLING. (see Otto, Allen and Brackett) . . . . .	21*
BRANSON, DOROTHY S. (see Ackert, Branson, Ameel and Riedel) . . . . .	26*
BROWN, HAROLD W. The present status of the filariasis problem . . . . .	17
<i>Bunostomum trigonocephalum</i> , cause of anemia in lambs . . . . .	18*
CABLE, R. M. AND RICHARD A. MCLEAN. Motion picture of <i>Cercaria clausii</i> Monticelli, a marine rattenkönig larval trematode from the West Coast of Florida . . . . .	8*
<i>Cercaria clausii</i> Monticelli, a marine rattenkönig larval trematode from the West Coast of Florida . . . . .	8*
<i>Chabertia ovina</i> , further notes on . . . . .	6*
Coccidiosis infection in chickens, effect of aryl arsonic acids on . . . . .	3*
CULBERTSON, JAMES T. (see Rose, Culbertson and Molloy) . . . . .	39*
CULBERTSON, JAMES T. AND HARRY M. ROSE. Agglutination of microfilariae from <i>Litosomoides carinii</i> of the cotton rat by normal serums of man and other animals . . . . .	23*
DAVIS, LEONARD REID. (see Hammond, Davis, Bowman and Simms) . . . . .	4*
<i>Dero limosa</i> , infected with <i>Adelina deronis</i> , culture of . . . . .	16*
DOVE, WALTER E. Development of louse powders for the armed forces . . . . .	18
DYER, R. E. Distribution and control of the fevers of the typhus group . . . . .	17
<i>Eimeria bovis</i> , life cycle in calves . . . . .	4*
<i>Eimeria tenella</i> , effects of sulphur drugs on . . . . .	1*
<i>Eimeria tenella</i> , infections in poultry, sulfamethazine in treatment of . . . . .	2*
<i>Endamoeba histolytica</i> , growth rates in cultures . . . . .	19*
EWING, HENRY E. The trombiculid mites (chigger mites) and their relation to disease . . . .	40*
FELSENFIELD, OSCAR. On the staining of protozoa in human blood and feces . . . . .	11*
Filariasis, an <i>in vitro</i> method for bio-assay of chemotherapeutic agents in . . . . .	39*
HAMMOND, DATUS M., LEONARD REID DAVIS, GEORGE W. BOWMAN AND BENNETT T. SIMMS. Experimental studies on the life cycle of <i>Eimeria bovis</i> in calves . . . . .	4*
HARWOOD, PAUL D. (see McMullen, Van Volkenberg and Harwood) . . . . .	31*
HAUSCHKA, THEODORE S. The continuous culture of pure clones of <i>Dero limosa</i> infected with <i>Adelina deronis</i> . . . . .	16*
HAWKINS, PHILIP A. Sulfamethazine in the treatment of <i>Eimeria tenella</i> infections in poultry . . . . .	2*
Helminths, common names of . . . . .	31*
Helminths, effects on growing chickens . . . . .	27*
HINSHAW, W. R. (see MacNeil and Hinshaw) . . . . .	15*
<i>Histoplasma capsulatum</i> , its biochemical and immunological properties . . . . .	9*

(\* refers to abstract number rather than page)

<i>Hymenolepis nana</i> var. <i>fraterna</i> , effect of alcoholism on resistance to .....	29*
LARSH, JOHN E. Alcoholism in mice and its effect on natural and acquired resistance to <i>Hymenolepis nana</i> var. <i>fraterna</i> .....	29*
Life cycle of <i>Eimeria bovis</i> in calves, experimental studies on .....	4*
<i>Litosomoides carinii</i> , agglutination of microfilariae by sera .....	23*
LUCKER, JOHN T. AND ELEANOR M. NEUMAYER. The production of anemia in lambs by hookworms, <i>Bunostomum trigonocephalum</i> .....	18*
LUTTERMOSER, GEORGE W. The possibility of eliminating the schistosome snail, <i>Australorbis glabratus</i> , from canals by controls of water flow .....	34*
MACNEIL, E. AND W. R. HINSHAW. A blood parasite of the turkey .....	15*
MAYFIELD, ORLEY J. (see Morehouse and Mayfield) .....	3*
MAHEW, ROY L. The effects of nematode infections during the prepatent period on the calf ..	28*
MCINTOSH, ALLEN (see Price and McIntosh) .....	13*
MCLEAN, RICHARD A. (see Cable and McLean) .....	8*
McMULLEN, DONALD B., H. L. VAN VOLKENBERG AND PAUL D. HARWOOD. Common names of helminths .....	31*
Microfilariae of <i>Litosomoides carinii</i> , agglutination by sera .....	23*
MOLLOY, ELEANORA (see Rose, Culbertson and Molloy) .....	39*
MOREHOUSE, NEAL F. AND ORLEY, J. MAYFIELD. The effect of some aryl arsonic acids on experimental coccidiosis infection in chickens .....	3*
MORGAN, BANNER BILL. Inoculations of <i>Trichomonas foetus</i> (Protozoa) in heifers .....	20*
<i>Necator americanus</i> , action of sera on infective larvae .....	21*
Nematode infections, effect on the calf .....	28*
Nematodes of swine, effects of skim milk on .....	38*
<i>Neoechinorhynchus emydis</i> (Acanthocephala), physiological responses to various solutions ..	5*
NEUMAYER, ELEANOR M. (see Lucker and Neumayer) .....	18*
NEWTON, WALTER L. AND WILLARD H. WRIGHT. Tests to determine the ability of species of domestic mosquitoes to transmit <i>Wuchereria bancrofti</i> .....	22*
NIGRELLI, ROSS F. Trypanosomes of North American amphibians .....	14*
OLIVER-GONZÁLES, JOSÉ. Cross reactions between polysaccharides from various animal parasites .....	24*
OLSEN, O. WILFORD. Critical tests with a hexachloroethane-bentonite-water suspension as a fasciolicide in cattle .....	30*
OTTO, GILBERT F., EVELYN ALLEN AND STERLING BRACKETT. The action of Negro and white sera on infective hookworm ( <i>Necator americanus</i> ) larvae .....	21*
Paramphistomes of North American domestic ruminants .....	13*
Parasitology in relation to the war symposium, Cleveland, Ohio, program of .....	5
Plethodontidae (Amphibia-Caudata), the parasites of .....	35,* 36*
Polysaccharides from various animal parasites, cross reactions between .....	24*
<i>Porocephalus crotali</i> Humbolt, occurrence of, in rattlesnakes of southwestern Oklahoma ....	12*
PRICE, EMMETT W. AND ALLEN MCINTOSH. Paramphistomes of North American domestic ruminants .....	13*
Proteidae (Amphibia-Caudata), the parasites of .....	37*
Protozoa, incidence of intestinal, in mental patients and in cases showing symptoms of amoebic dysentery .....	10*
Protozoa, staining of, in human blood and feces .....	11*
REARDON, LUCY V. (see Rees and Reardon) .....	19*
REES, CHARLES W. AND LUCY V. REARDON. Comparative growth rates of amoebae and bacteria in cultures of <i>Endamoeba histolytica</i> and organism <i>t.</i> .....	19*
REID, W. MALCOLM. The effects of host starvation on worm elimination and glycogen depletion with the nematode, <i>Ascaridia galli</i> .....	25*
RELLER, HELEN (see Tsuchiya and Reller) .....	17*
RIEDEL, B. B. (see Ackert, Branson, Ameel and Riedel) .....	26*

(\* refers to abstract number rather than page)

RIPSOM, CHARLES A. Effects of various sulpha drugs on the protozoan parasite, <i>Eimeria tenella</i> .....	1*
ROSE, HARRY M. (see Culbertson and Rose) .....	23*
ROSE, HARRY M., JAMES T. CULBERTSON AND ELEANORA MOLLOY. An <i>in vitro</i> method for the bio-assay of chemotherapeutic agents in filariasis .....	39*
ROSS, ELIZABETH L. (see Van Cleave and Ross) .....	5*
SCHIEFF, GEORGE J. <i>Histoplasma capsulatum</i> , its biochemical and immunological properties ..	9*
SCHWARTZ, BENJAMIN. Parasites as factors in production of meat and other animal products in wartime .....	18
SEITNER, PHILIP G. Studies on a new xiphidiocercaria of the virgula type with notes on the life history .....	7*
SELF, J. TEAGUE. The occurrence of the pentastomid, <i>Porocephalus crotali</i> Humbolt, in rattlesnakes of southwestern Oklahoma .....	12*
SIMMS, BENNETT T. (see Hammond, Davis, Bowman and Simms) .....	4*
Sirenidae (Amphibia-Caudata), the parasites of .....	37*
SMITH, VIVIAN S. A reaction of rat serum to embryonated eggs of <i>Trichosomoides crassicauda</i> .....	32*
SMITH, VIVIAN S. Studies on the relationship of <i>Trichosomoides crassicauda</i> infection in rats to mucoid calculi of the urinary bladder .....	33*
SPINDLER, L. A. AND HARRY E. ZIMMERMAN, JR. Effect of skim milk on infections of ascarids, whipworms and nodular worms in swine .....	38*
Sulpha drugs, effects on protozoan parasite, <i>Eimeria tenella</i> .....	1*
Sulfamethazine in the treatment of <i>Eimeria tenella</i> infections in poultry .....	2*
Symposium on parasitology in relation to the war, Cleveland, Ohio, program of .....	5
THRELKELD, W. L. Further notes on <i>Chabertia ovina</i> .....	6*
<i>Trichomonas foetus</i> , inoculations in heifers .....	20*
<i>Trichosomoides crassicauda</i> , infection in rats and mucoid calculi of urinary bladder .....	33*
<i>Trichosomoides crassicauda</i> , reaction of rat serum to embryonated eggs of .....	32*
<i>Trichostrongylus</i> sp. and identification of ova .....	17*
Trypanosomes of North American amphibians .....	14*
TSUCHIYA, H. AND HELEN RELLER. A case of <i>Trichostrongylus</i> sp. with notes on the identification of ova .....	17*
VAN CLEAVE, HARLEY J. AND ELIZABETH L. ROSS. Physiological responses of <i>Neoechinorhynchus emydis</i> (Acanthocephala) to various solutions .....	5*
VAN VOLKENBERG, H. L. (see McMullen, Van Volkenberg and Harwood) .....	31*
WALTON, ARTHUR C. The parasites of the Plethodontidae (Amphibia-Caudata) III and IV .....	35*, 36*
WALTON, ARTHUR C. The parasites of the Proteidae and the Sirenidae (Amphibia-Caudata) ..	37*
WISSEMAN, C. L. (see Ackert and Wisseman) .....	27*
WRIGHT, WILLARD H. (see Newton and Wright) .....	22*
<i>Wuchereria bancrofti</i> , transmission by domestic mosquitoes .....	22*
Xiphidiocercaria of the virgula type .....	7*
YOUNG, VIOLA M. The incidence of intestinal protozoa in mental patients and in cases showing symptoms of amoebic dysentery .....	10*
ZIMMERMAN, HARRY E. (see Spindler and Zimmerman) .....	38*